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# **The Extra-Pulmonary Effects of Chronic Obstructive Pulmonary Disease (COPD)**

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degree of Doctor of Philosophy

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# **Abstract**

## **Rationale**

Cardiovascular disease (CVD) is a leading cause of mortality in patients with COPD. Aortic stiffness, measured using aortic pulse wave velocity (PWV), an independent, non-invasive, predictor of CV risk; and inflammatory markers are increased in COPD. Screening tools for community based identification of increased CVD risk, and a proactive approach to addressing primary prevention of CVD is needed. Statins modulate aortic stiffness and are anti-inflammatory, but are not currently used for primary prevention in COPD.

## **Objectives**

Proof of principle double-blind Randomised Control Trial (RCT) to determine if six weeks simvastatin 20mg od reduces aortic stiffness, systemic and airway inflammation in COPD. Cross-sectional pilot study comparing a non-invasive measure of oxidative stress (skin "AGE") in COPD and controls, to lung function and aortic stiffness.

## **Methods**

Stable patients (n=70) were randomised to simvastatin or placebo treatment. Pre- and post-treatment aortic stiffness, blood pressure, spirometry, circulating inflammatory mediators and lipids were measured; airway inflammatory markers were performed where possible. Predefined subgroup analysis was performed where baseline aortic PWV >10m/s.

For the cross-sectional study stable COPD patients (n=84) and controls (n=36) had lung function, arterial stiffness and skin AGE measured.

## **Results**

In the RCT the active group achieved significantly lower total cholesterol, but no significant drop in aortic PWV compared to placebo group:  $-0.7(95\%CI -1.8,0.5)m/s$ ,  $p=0.24$ ; or inflammatory markers. In those with higher baseline aortic PWV,  $n=22$ , aortic PWV improved in the active group compared to placebo:  $-2.8(-5.2,-0.3)m/s$ ,  $p=0.03$ .

Skin AGE was increased in COPD compared to controls, inversely related to lung function, and directly related to aortic stiffness.

## **Conclusions**

We could not detect any significant difference in the change in aortic PWV in patients with COPD taking simvastatin compared to placebo. We did, however, report a significant and clinically relevant reduction in aortic PWV in those with high baseline aortic stiffness, suggesting a potential for statins to reduce CV morbidity in high risk individuals. The pilot cross-sectional study suggests there is an indication to assess the potential role of skin AGE in patients with COPD as a non-invasive measure of CV risk.

## **Author's Publications**

### **Publications**

John, M. Cockcroft, J.R. McKeever, T.M. Coward, W.R. Shale, D.J. Johnson, S.R. Thornton, J.G. Harrison, T.W. Knox, A.J. and Bolton, C.E. *The Cardiovascular and Inflammatory Effects of Simvastatin Therapy in Patients with COPD*. Submitted.

John M, Hussain S, Prayle A, Simms R, Cockcroft JR and Bolton CE. *Target renal damage: the microvascular associations of increased aortic stiffness in patients with COPD*. Respiratory Research 2013, 14:31.

### **Peer-reviewed Abstracts**

John, M. Alhaddad, M. Hussain, S. and Bolton, C.E. *Advanced Glycation Endproducts and Lung Function in COPD* European Respiratory Society, 2014.

John, M. Cockcroft, J.R. McKeever, T.M. Harrison, T.W. Shale, D.J. Thornton, J.G. Knox, A.J. and Bolton, C.E. *Cardiovascular Effects of Statin Therapy on Arterial Stiffness in Patients with COPD: A Double Blind Randomised Controlled Trial*. Thorax 2013; 68: Suppl3 A133.

John, M. Knox, A.J. McKeever, T.M. Meakin, G. Bailey, H. Cockcroft, J.R. Shale, D.J. Harrison, T.W. and Bolton, C.E. *The Effects of Statin Therapy on Inflammatory Markers in Patients with COPD: A Double Blind Randomised Controlled Trial*. Thorax 2013; 68: Suppl3 A16-17. Spoken presentation.

John, M. Hussain, S and Bolton, C.E. *Cardiovascular Risk Prediction Scores - missing a "COPD" factor?* AJRCCM-Abstract Issue, 2013. 187: A2411

John, M. Hussain, S. Simms, R. Cockcroft, J.R. Prayle, A and Bolton, C.E.  
*Glomerulopathy, microvascular damage and aortic stiffness in patients with COPD*  
2012 ERS **P1911**

John, M. Hussain, S. And Bolton, C.E. *The Association Of Sputum Production And Arterial Stiffness In Chronic Obstructive Pulmonary Disease (COPD)* AJRCCM-Abstract Issue, 2012. 185: 27945

John, M. Hussain, S. Selvarajah, S. Sayers, I. Hall, I.P. Fairclough, L. and Bolton, C.E.  
*Increased Advanced Glycation End Products in Patients With Chronic Obstructive Pulmonary Disease (COPD)* Thorax 2011; 66: Suppl4 A84

John, M. Hussain, S. Gerrard-Tarpey, W. Smith, K and Bolton, C.E. *Advanced Glycation End Products in Chronic Obstructive Pulmonary Disease (COPD)* British Association for Lung Research meeting, 2011.

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## **Abbreviations**

6MWT	Six Minute Walk Test
$\alpha$ 1AT	Alpha-1 antitrypsin
AAA	Abdominal Aortic Aneurysm
ADO	Age Dyspnoea Obstruction
AECOPD	Acute Exacerbation of Chronic Obstructive Pulmonary Disease
AF	Atrial Fibrillation
AGE	Advanced Glycation Endproducts
AIx	Augmentation Index
ALT	Alanine Aminotransferase
ANOVA	Analysis Of Variance
ATS	American Thoracic Society
BIA	Bioelectrical Impedance Analysis
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Blood Pressure
bpm	beats per minute
BODE	BMI, Obstruction, Dyspnoea, Exercise (an index)
BOLD	Burden of Obstructive Lung Disease
BTS	British Thoracic Society
CAD	Coronary Artery Disease

CAT	COPD Assessment Test
CEL	N-(carboxyethyl)lysine
CML	carboxymethyl-lysine
CO	Carbon monoxide
COHb	Carboxyhaemoglobin
COPD	Chronic Obstructive Pulmonary Disease
CPK	Creatine Phosphokinase
CRP	C-Reactive Protein
CT	Computed Tomography
CTU	Clinical Trials Unit
CV	Cardiovascular
DEXA	Dual Energy X-ray Absorptiometry
ECG	Electrocardiogram
ECRHS	European Community Respiratory Health Survey
ELISA	Enzyme-Linked Immunosorbent Assay
ERS	European Respiratory Society
FeNO	Fractional Exhaled Nitric Oxide
FEV <sub>1</sub>	Forced Expired Volume in 1 second
FFM	Fat Free Mass
FFMI	Fat Free Mass Index
FM	Fat Mass
FMI	Fat Mass Index

FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
Gamma GT	Gamma-glutamyltransferase
GOLD	Global Initiative on Obstructive Lung Disease
GP	General Practitioner
GWAS	Genome Wide Association Study
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
HRCT	High Resolution Computed Tomography
HRQoL	Health Related Quality of Life
ICAM-1	Intercellular Adhesion Molecule-1
ICS	Inhaled Corticosteroids
IGF-1	Insulin-like Growth Factor-1
IHD	Ischaemic Heart Disease
IL	Interleukin
iNOS	inducible Nitric Oxide Synthase
IRAS	Integrated Research Application System
Kg	Kilogram
L	Litre
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
m	metre

MAP	Mean Arterial Pressure
mg	milligram
MDI	Metered Dose Inhaler
MHRA	Medicines and Healthcare products Regulatory Agency
MMP	Matrix Metalloproteases
MRC	Medical Research Council
NHS	National Health Service
NICE	National Institute for health and Clinical Excellence
nm	nanometer
NO	Nitric Oxide
NOS	Nitric oxide synthase
NRRU	Nottingham Respiratory Research Unit
OCS	Oral corticosteroids
PaCO <sub>2</sub>	Arterial partial pressure of carbon dioxide
PaO <sub>2</sub>	Arterial partial pressure of oxygen
PCRN	Primary Care Research Network
PEF	Peak Expiratory Flow
PIC	Patient Identification Centres
PIS	Participant/Patient Information Sheet
PP	Pulse Pressure
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity

R&D	Research and Development
RAGE	Receptor for Advanced Glycation End-products
RCT	Randomised Control Trial
REC	Research Ethics Committee
RHF	Right-sided Heart Failure
RV	Residual Volume
SD	Standard Deviation
SGRQ	St George's Respiratory Questionnaire
SmPC	Summary of Product Characteristics
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedure
SpO <sub>2</sub>	Peripheral oxygen saturation
SPSS	Statistical Package for the Social Sciences
sRAGE	soluble Receptor for Advanced Glycation End-products
TLC	Total Lung Capacity
TNF- $\alpha$	Tumour Necrosis Factor- $\alpha$
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation

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# **Chapter 1**

## **Introduction**

# **1 Introduction**

## **1.1 Definition**

Chronic Obstructive Pulmonary Disease (COPD) is a term used to encompass a spectrum of disease from chronic bronchitis and emphysema, usually as a result of a significant tobacco smoking exposure. Both chronic bronchitis and emphysema can be present in isolation, without the presence of COPD. The key factor for a diagnosis of COPD is non-reversible airflow obstruction, where the Global Initiative on Obstructive Lung Disease (GOLD) initially defined COPD as “a disease state characterised by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases” (Pauwels et al., 2001). More recently the definition has been extended to include extra-pulmonary effects and comorbidities which may impact on the severity of the condition (Decramer et al., 2013). As the condition progresses it can result in chronic (type II) respiratory failure (Raherison and Girodet, 2009).

The heterogeneity of COPD has chronic bronchitis at one end of the spectrum and emphysema at the other end. Chronic bronchitis is defined clinically as a chronic productive cough for at least three months of two consecutive years, in the absence of other diseases recognised as causing sputum production (MRC, 1965). Whereas emphysema is defined pathologically as the permanent, abnormal enlargement of the air spaces distal to the terminal bronchioles, accompanied by destruction of their walls and without obvious fibrosis (ATS, 1995), leading to the irreversible loss

of elastic recoil with collapsing of the small, distal airways (Snider, 1985). As these changes can occur without resulting in airflow limitation, a diagnosis of chronic bronchitis or emphysema can be made without meeting the definition of COPD. The obstructive ventilatory impairment associated with chronic bronchitis is attributed to increased inflammation, changes in the epithelium and increased secretions in the small airways (Snider, 1985). As tobacco smoking is the main contributing factor to both chronic bronchitis and emphysema the two conditions often occur simultaneously, giving rise to the disorder chronic obstructive pulmonary disease (Snider, 1985).

## **1.2 Epidemiology**

According to the World Health Organisation (WHO) COPD is the fourth leading cause of death in the world, and it is expected to become the third leading cause of death worldwide by 2020 (Raherison and Girodet, 2009, Murray and Lopez, 1997). COPD is the second leading cause of smoking attributable mortality, behind cardiovascular disease (Ezzati and Lopez, 2003). It is estimated that 2.75 million (4.8%) deaths/year worldwide are attributable to COPD, with 30,000 of these deaths/year in the United Kingdom (UK) (Raherison and Girodet, 2009). COPD is a growing problem in both the developed and developing countries throughout the world. The prevalence statistics of COPD vary widely depending on the definition and classification methods, as recently demonstrated by the Burden of Obstructive Lung Disease (BOLD) (Buist et al., 2007). Over a million people are diagnosed with COPD in the UK (Raherison and Girodet, 2009), with a further 2 million undiagnosed. Previously the prevalence of COPD has been greatest in males, due to

smoking habits, but recently the prevalence in females has increased, particularly in young females; and in the United States of America (USA) the prevalence and deaths from COPD in females has now exceeded that of males (Mannino et al., 2002).

COPD has a negative impact on the subjects' quality of life and is a huge economic burden. The disabling effects of COPD vary depending on the severity of the condition and the susceptibility of the subject (Raheison and Girodet, 2009). Economic burden is related to both the impact on the health service including hospital admissions and ambulatory care and the inability to work (Raheison and Girodet, 2009). The most significant healthcare cost is that associated with exacerbations and hospitalisations (Chapman et al., 2006). Historical annual direct costs have been estimated to be over £819 per patient per year in the U.K. (Britton, 2003), with total direct costs to the National Health Service (NHS) in England and Wales of over £4.9 million and indirect costs of £9.82 million (NICE, 2010a). At present the cost to employers is high. Health campaigns aimed at highlighting risk factors could lead to a reduction of new cases of COPD, reducing hospitalisation and improving out-patient services (Nurmagambetov et al., 2006).

### **1.3 Aetiology**

There is a five-fold increase in COPD diagnoses in those with tobacco exposure, however there are also environmental and genetic causes, demonstrated by 4% of people world-wide with COPD being never smokers (Raheison and Girodet, 2009). Risk factors for COPD can be classed as either host factors, for example age, gender and genetics; or acquired factors such as social and economic factors, passive and

active smoking, occupational and environmental exposure and infections (Raherison and Girodet, 2009).

### **1.3.1 Host Factors**

#### *1.3.1.a Gender*

A study looking at a Caucasian population of either current or ex-smokers in Norway with 954 patients with COPD and 955 controls; reported that there are gender differences, which appear to make females more susceptible to the effects of cigarette smoking and consequent lung damage (Sorheim et al., 2010). Females with COPD are younger, have a lower pack year smoking history compared with males, have more severe disease than males and an earlier age of disease onset (Sorheim et al., 2010). More females than males were current smokers, however more males than females had reported occupational exposure to dust or gas (Sorheim et al., 2010). Females may be more genetically susceptible to the detrimental effects of smoking because of the gender differences in smoke metabolism (Ben-Zaken Cohen et al., 2007). In a study with 278 participants Benowitz *et al.* investigated nicotine metabolism, and concluded females have a faster plasma clearance of nicotine and cotinine and higher nicotine to cotinine conversion when compared to males (Benowitz et al., 2006). Animal studies have also demonstrated gender differences in the response to smoke. The increased levels of toxic nicotine metabolites in females could be responsible for the increased inflammatory reaction and generation of oxidative airway stress (Forkert et al., 1996). As female airways are anatomically smaller than males, each cigarette may therefore also represent proportionally greater exposure, leading females to

develop more airway-based COPD rather than the emphysematous phenotype which is seen more in males (Han et al., 2007). Hormonal contribution (Becklake and Kauffmann, 1999), differing occupational exposure, second-hand smoke, inhalation pattern and subjective symptom reporting are other possible explanations for gender differences (Sorheim et al., 2010).

#### *1.3.1.b Genetics*

As not all smokers develop COPD there is evidence for other contributing factors to developing the condition, such as genetic predisposition (Wan and Silverman, 2009). The most described genetic disposition is Alpha-1 antitrypsin ( $\alpha_1$  AT) deficiency – a common but often under-diagnosed autosomal, co-dominant hereditary condition (Ioachimescu and Stoller, 2005), representing between 1-3% of people diagnosed with COPD (Raherison and Girodet, 2009).  $\alpha_1$  AT is a serine protease inhibitor protein (anti-protease) (Bartels et al., 2009), with its deficiency shown to be caused by mutations within the SERPINA1 (SERine Proteinase Inhibitor A1) gene. The link between  $\alpha_1$  AT deficiency and respiratory dysfunction was first described in 1963 by Laurell and Eriksson (Laurell, 1963), with low levels of  $\alpha_1$  AT now quantified as being less than 11 micromol/L (Ranes and Stoller, 2005).  $\alpha_1$  AT deficiency increases the risk of developing panacinar emphysema at an earlier age than that which COPD usually presents; and is also associated with bronchiectasis (Parr et al., 2007) and liver dysfunction (Strange et al., 2006).

If the balance between proteases and anti-proteases is disturbed, as with  $\alpha_1$  AT deficiency, anti-proteases no longer inhibit the actions of proteases, thus the lungs are not protected. Proteases, for example neutrophil elastase, will then destroy

lung tissue and results in alveolar wall destruction. Increasing levels of unopposed neutrophil elastase, an inflammatory protease that gets released in response to pathogens entering the lungs (Lee and Downey, 2001), results in elastin degrading normal host lung tissue (Ranes and Stoller, 2005).

$\alpha_1$  AT is a polymorphic molecule with approximately 100 alleles identified, of which more than 30 have been identified as resulting in  $\alpha_1$  AT deficiency (ATS/ERS, 2003). The most common deficient  $\alpha_1$  AT allele is the 'Z' variant – people who are PI\*ZZ homozygotes have plasma  $\alpha_1$  AT levels approximately 15% of normal; therefore these people are the ones most at risk of developing lung disease. Smoking acts synergistically with factors that decrease  $\alpha_1$  AT. Other known genetic causes include matrix metalloproteinases, specifically MMP-12 (Hunninghake et al., 2009), MMP-1 and MMP-9 (Molfino, 2004).

### **1.3.2      Acquired Factors**

#### *1.3.2.a Social and economic factors*

Social and economic conditions contribute to the risk of developing COPD, with people living in poor socio-economic circumstances more at risk of poor lung function and developing the condition (Gershon et al., 2011). Residential deprivation was reported from 30,445 individuals using the Townsend score as an index to identify material deprivation. Social deprivation can result in overcrowding within houses, which could increase stress and therefore be an influencing factor for smoking uptake; whereas education may increase health-related knowledge and therefore influence people to lead healthier and more active lifestyles. People living in deprived areas were found to have one and a half to two times the



likelihood of being a current smoker compared to those living in more affluent areas (Shohaimi et al., 2003). A cross sectional study with 22,675 adult participants from the general population found that after controlling for smoking history where 67% of males and 44% of the females were either current or ex-smokers, social class, education and living in areas of deprivation independently predicted impaired lung function (Shohaimi et al., 2004). This is likely through a number of variables such as smoking habits, diet, occupational and environmental exposure and lifestyle which are discussed below.

#### 1.3.2.a.i Smoking

The main risk factor especially in the developed world for developing COPD is active smoking. Smoking habits vary depending on the physical environment, with Shohaimi *et al.* describing social class and educational background as predictors for smoking uptake (Shohaimi et al., 2003).

There is a positive correlation between current and ex-smokers and COPD mortality (Enstrom and Kabat, 2003, Eisner et al., 2006). There have been many suggested mechanisms as to how and why cigarette smoking causes such damage to lung tissue. Cigarette smoke stimulates inflammatory cell recruitment into the lung parenchyma, leading to the release of elastolytic proteases, which then destroy lung extracellular matrix and result in air space enlargement – these pathological changes represent the changes that occur as emphysema develops (Shapiro, 2003).

Passive smoke exposure can increase the risk of getting COPD (Robbins et al., 1993). Passive smoking, in addition to active smoking, influences the outcome in COPD patients, and causes greater COPD severity and poorer health status (Eisner et al.,

2006). The smoke released from a lit cigarette together with the smoke exhaled from a smoker forms the gaseous mixture inhaled by a passive smoker (Janson, 2004), and contains many potent respiratory irritants (Janson et al., 2001). The European Community Respiratory Health Survey reported that passive smoking also increased respiratory symptoms such as nocturnal and exercise related chest tightness and breathlessness (Janson et al., 2001). There are high rates of passive workplace smoke exposure in Europe, whereas New Zealand, Australia and America have low workplace smoke exposure (Janson et al., 2001). Cotinine, a metabolite of nicotine, and measure of tobacco was increased in habitants of those living with a smoker. A link between cotinine and forced expired volume in 1 second ( $FEV_1$ ) was also reported, with a 105ml difference in  $FEV_1$  between subjects with the highest and lowest passive tobacco smoke exposure (Carey et al., 1999). Xu *et al.* reported passive smoking related to a small decline in lung function results, with an approximate decline in  $FEV_1$  of 100mls and Forced Vital Capacity (FVC) of 150mls (Xu and Li, 1995).

#### 1.3.2.a.ii Occupation

Occupation offers a significant risk factor to developing COPD and has wide variation depending on geographical location, as illustrated by studies in Norway finding associations between airflow obstruction and exposure to ammonia, metal fumes and anhydrides; in contrast to New Zealand finding associations with cleaners, laboratory technicians and construction workers (Hnizdo et al., 2002). Occupational exposure to both organic and inorganic dust and chemical agents increases the risk of developing COPD, with increased odds ratios for

transportation-related occupations, machine operators and construction trades (Hnizdo et al., 2002).

#### 1.3.2.a.iii Environmental exposure

Air pollution increases the likelihood of a subject developing COPD and can trigger exacerbations. Particles with a diameter of 100nanometers (nm) or less are defined as ultrafine particles (Donaldson et al., 2001), with diesel soot being the most numerous (MacNee and Donaldson, 2000). Cigarette smoking and environmental pollution causes lung inflammation, resulting in a systemic inflammatory response, which leads to endothelial activation and changes in atherosclerotic plaques typical of plaque instability; and these vascular changes contribute to the increased cardiovascular (CV) morbidity and mortality in people with COPD (van Eeden et al., 2005). Developing countries have increased risk from pollution inside homes, where smoke from cooking or heating accumulates in badly ventilated houses (Raherison and Girodet, 2009). Approximately 50% of the population use biomass fuel as their primary source of energy, but in developing countries this increases to around 90% (Bruce et al., 2000). A considerable amount of the biomass smoke constituents are known to be toxic or are an irritant to the respiratory system (Hu et al., 2010).

#### 1.3.2.b *Early life factors*

Early life environment may be another potential factor for impaired lung function and increased risk of developing COPD in later life. Lower birth weight has been associated with reduced adult lung function, where mean FEV<sub>1</sub> at 59-70 years of age is higher by 0.06L for every 1lb increase in birth weight – as recorded at the

time by a health visitor, independent of smoking habit and social class (Barker et al., 1991). A relationship between lower respiratory tract infections in early life and subsequent development of COPD has been shown; and Bronchitis or pneumonia in childhood has been related to a reduced FEV<sub>1</sub> (Shaheen et al., 1995). Those with a lower socio-economic status during childhood were more likely to have a poor diet, and this poor nutrition impacts upon growth. An inverse relationship between height and COPD risk was linked with socioeconomic status, particularly in younger adults (Ward and Hubbard, 2009). A cross sectional analysis of lung function data and information from early life has highlighted the impact of childhood disadvantage – maternal, paternal or childhood asthma, respiratory infections and maternal smoking - as being as significant as that of heavy smoking (Svanes et al., 2010). Reducing early childhood lung growth could prevent people reaching their potential maximum lung function (Barker et al., 1991), and this lowered lung function has no rectification with age; but as socioeconomic standards are improving, the effect of early life influences may become less important (Ward and Hubbard, 2009).

## **1.4 Pathology and Pathophysiology**

The characteristic pathological changes associated with COPD occur in the central and peripheral airways, lung parenchyma and pulmonary vasculature (Pauwels et al., 2001). Enlargement of the mucous secreting glands and an increase in the number of goblet cells are responsible for the hyper-secretion of mucous associated with the chronic bronchitic element of COPD (Snider, 1985). Chronic bronchitis is characterised by hyperplasia and hypertrophy of the sub-mucosal

bronchial glands, which causes mucous hyper-secretion (Snider, 1985). The emphysematous component of COPD results in the destruction of lung parenchyma, causing dilation and destruction of respiratory bronchioles. The destruction of alveoli is a key component; and subsequent loss of elastic recoil with the consequent collapse of small, poorly supported, distal airways (Snider, 1985). Associated physiological changes include ciliary dysfunction, pulmonary hyperinflation, gas exchange limitations, pulmonary hypertension and eventually cor pulmonale (MacNee, 1994).

Expiratory airflow obstruction arises as a consequence of permanent parenchymal destruction and airway dysfunction. People with COPD have a faster rate of decline in their lung function (Mannino and Davis, 2006). Donaldson *et al.* demonstrated that in 109 patients with COPD, the ones with more frequent exacerbations had a faster rate of decline in FEV<sub>1</sub> and Peak Expiratory Flow (PEF) (Donaldson et al., 2002). With emphysematous changes the lungs elastic recoil pressure is reduced, and subsequently the driving pressure for expiration is reduced.

The bronchoconstricted airways in COPD have an increased resistance (O'Donnell and Laveneziana, 2006). Residual Volume (RV) is often the first static lung volume to increase, and is therefore the first sign of increasing airway closure. Subsequently, Functional Residual Capacity (FRC) increases as a result of expiratory airflow obstruction and alterations in the static lung mechanics (O'Donnell and Laveneziana, 2006). Dynamic hyperinflation has many effects upon the respiratory system. It leads to increases in the elastic load on the inspiratory muscles, and consequently increases both the work of breathing and the oxygen cost of

breathing (O'Donnell et al., 1997). Tidal volume expands so there is a low inspiratory reserve volume, and becomes fixed on the upper less compliant extreme of the pressure-volume relationship. This results in inspiratory muscle weakness by maximally shortening muscle fibres in the diaphragm (Sinderby et al., 2001) and increased breathing frequency, leading to significant reductions in lung compliance (O'Donnell and Laveneziana, 2006). It can also cause a reduced ability to modify tidal breathing during exertion, which then also leads to mechanical ventilation limitation (O'Donnell et al., 2002). The combination of effects as a result of dynamic hyperinflation contributes to the symptoms of dyspnoea and exercise limitation experienced by patients with COPD (O'Donnell and Laveneziana, 2006).

Peripheral airway changes include airway inflammation, the development of fibrosis and smooth muscle hypertrophy, which all contribute to the thickening of the airway wall; and goblet cell metaplasia can lead to mucus plugging and facilitate the occlusion of lumen (Saetta et al., 1985, Saetta et al., 2001). Central airway changes include an increase in the number of macrophages and T-lymphocytes in the airway wall, and an increase in the number of neutrophils in the airway lumen (Di Stefano et al., 1996).

An early pathological feature of COPD is the vascular changes. The intima of pulmonary vessels becomes thickened and there is an increase in smooth muscle and infiltration of the vessel wall by inflammatory cells (Peinado et al., 1999).

The surface epithelium of the central airways are infiltrated by inflammatory cells (Pauwels et al., 2001), characteristically neutrophils, macrophages, B cells, lymphoid aggregates, and CD8<sup>+</sup> T-Lymphocytes (Chung, 2005). There are increased

levels of CD8<sup>+</sup> T- Lymphocytes, and an increased smooth muscle area in the peripheral airways of smokers with symptomatic COPD in comparison with asymptomatic smokers with normal lung function (Saetta et al., 1998). The increased levels of CD8<sup>+</sup> T- Lymphocytes in the central airways are also found in the peripheral airways. It is this peripheral airways site which is responsible for the chronic airflow limitation and hence obstructive ventilatory impairment demonstrated in smokers (Saetta et al., 1998).

Airway smooth muscle produces inflammatory cytokines, proteases and growth factors, which contribute to the airway wall remodelling process. Airway wall remodelling causes airway thickening and airflow obstruction, and occurs in the small airways through the processes of tissue repair and epithelial metaplasia. This airway remodelling could also include the release of growth factors from inflammatory cells (Chung, 2005). Chronic inflammation within the peripheral airways leads to the continuous cycle of cell injury and repair of the airway wall. This repairing process of the airway wall then leads to structural remodelling, causing the airway lumen to become narrowed and results in fixed obstruction as a consequence of increased collagen content and scar tissue formation (Pauwels et al., 2001). The pathogenesis of COPD is largely attributable to the important role of CD8<sup>+</sup> T- Lymphocytes and airway remodelling (Saetta et al., 1998).

Subjects with COPD also have a heightened persisting systemic inflammatory state. Levels of C-Reactive Protein (CRP), fibrinogen, leucocytes and Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) have been shown to be elevated in people with COPD (Gan et al., 2004), which is not attenuated with smoking cessation (Gan et al., 2004). Matrix

metalloproteinases (MMPs) are structurally related metalloendopeptidases, vital for homeostasis and extracellular matrix turnover in both health and disease (Lowrey et al., 2008). Circulating MMP-9 levels have been previously shown to be elevated in clinically stable patients with COPD compared to healthy controls (Bolton et al., 2009b). This increase in MMP-9 seen in patients with COPD could be due to neutrophil activation (Bolton et al., 2009b) or released from monocytes (Aldonyte et al., 2003).

There have been two proposed explanations for the associations between COPD and systemic inflammation. At first it was thought the inflammation from the lungs 'over-spilled' into systemic circulation, resulting in low-grade systemic inflammation. However, lack of correlation between plasma and sputum concentrations of soluble TNF receptors and Interleukin-8 (IL-8) suggests there is more to the inflammation process than the simple 'over spill' theory (Vernooy et al., 2002, Sapey et al., 2009). The other explanation is to consider COPD as a systemic inflammatory disease, where pulmonary inflammation and lung disease is one component of the multi-organ compromise (Barnes and Celli, 2009). A recent study by Rutten *et al.* has suggested a further significant contributor to systemic inflammation in stable moderate to severe COPD patients is abdominal fat mass. A positive association between plasma CRP and both abdominal fat mass (FM) and fat mass index (FMI) was demonstrated (Rutten et al., 2010).

## **1.5 Diagnosis**

There are several important stages in diagnosing COPD. These are primarily taking an accurate history including exposures, clinical examination and lung function



testing, usually spirometry (Raherison and Girodet, 2009). Spirometrically, COPD in the UK has until recently been confirmed as a forced expiratory volume in one second ( $FEV_1$ ) and forced vital capacity (FVC) ratio ( $FEV_1/FVC$  ratio)  $<0.7$  and an  $FEV_1 <80\%$  predicted (Gomez and Rodriguez-Roisin, 2002, NICE, 2004); however, in recent years recognition of milder airflow obstruction is sufficient for mild COPD when accompanied by symptoms (NICE, 2010a). Good quality spirometry measurements are key to aiding diagnosis and monitoring disease progression, requiring appropriately qualified and experienced health care professionals. A diagnosis of COPD is made using post bronchodilator spirometry results to alleviate misdiagnosis due to a reversible obstructive component as seen with chronic asthma (Pearson et al., 1997).

At diagnosis, in addition to spirometry, subjects should also have a chest x-ray, full blood count and have their Body Mass Index (BMI) calculated (NICE, 2010a). Subjects 40 years of age or older with a smoking history equivalent to 10 pack years or greater, presenting with cough and/or sputum production are at risk of developing COPD and a COPD diagnosis should be considered (Raherison and Girodet, 2009). COPD often remains hidden, with many individuals having undiagnosed airflow obstruction (Coultas et al., 2001), highlighting the need to be more pro-active in diagnosis. Bednarek *et al.* reported in an observational primary care study looking at current, ex- and never smokers that patients 40 years of age or older had a COPD prevalence of 9.3%, however only 19% of these had been diagnosed and treated before the study; demonstrating the significant airflow limitation and loss of lung function before diagnosis (Bednarek et al., 2008).

## 1.6 Investigations

### 1.6.1 Spirometry

The physiological measurement in lung function, which forms the basis of the spirometric assessment of airway obstruction, is FEV<sub>1</sub>, where with lower FEV<sub>1</sub> there is greater airflow limitation. This measure of expiratory airflow limitation has been found to strongly correlate with increased mortality, and is a risk factor for cardiovascular disease, stroke and lung cancer (Hole et al., 1996). The inverse relationship between FEV<sub>1</sub> and increased mortality as seen in COPD was also demonstrated in life-long non-smokers (Hole et al., 1996). FEV<sub>1</sub> is the key marker of respiratory function in monitoring the disease progression because it is objective and quantitative. Recognition that other factors to assess severity is needed, as it is debateable how useful just FEV<sub>1</sub> is at assessing disease severity and progression; it only accounts for airway obstruction, not other respiratory manifestations, neither does it account for any of the systemic effects of COPD, given that COPD is now recognised as having extra pulmonary manifestations (Oga et al., 2007). Currently, slowing the rate of decline of FEV<sub>1</sub> has only been achieved by smoking cessation (Hole et al., 1996).

#### *1.6.1.a Severity of airflow obstruction*

There have previously been several variations in the guidelines used to classify the severity of COPD. The first guidelines on the management of COPD in the UK were produced by the British Thoracic Society (BTS) in 1997 (Pearson et al., 1997). These guidelines suggested a three stage approach to categorising patients with either mild, moderate or severe COPD based on their FEV<sub>1</sub> values (Pearson et al., 1997).

These were replaced with the 2004 NICE guidelines which reclassified the percentages of FEV<sub>1</sub> used (NICE, 2004). Revised NICE 2010 guidelines now suggest a 4 stage approach based on post bronchodilator spirometry (NICE, 2010a); see Table 1.1.

In parallel with this, the GOLD strategy have a 4 stage approach (Decramer et al., 2013). Previous versions of GOLD guidelines had a GOLD 0 category, which was an at risk group of subjects with normal spirometry but having symptoms such as chronic cough and sputum production (Pauwels et al., 2001). Studies have highlighted that GOLD stage 0 was not applicable to detect at risk patients (Vestbo and Lange, 2002). This was a contentious issue because there are many in-patient admissions and out-patient appointments for the 'at risk' group of subjects because they are symptomatic, and no treatment recommendations exist for this GOLD 0 group of subjects other than smoking cessation (Joo et al., 2008). The GOLD groups are shown in Table 1.1, and have been used to classify airflow obstruction severity in my thesis. The ATS/ERS had previously published guidelines suggesting a 5 stage categorising process based on post bronchodilator spirometry, where the first stage is still an 'at risk' category with an FEV<sub>1</sub>/FVC ratio >0.7 and an FEV<sub>1</sub> ≥ 80% but incorporating risk factors (Celli and MacNee, 2004). See Table 1.2.

The latest GOLD guidelines also includes a four category (A, B, C and D) risk assessment strategy using symptoms as assessed by the modified MRC or CAT score, GOLD classification of airflow limitation and exacerbation history (Decramer et al., 2013).

It is clear that with so many differing guidelines published over many years on staging COPD, severity confusion can arise and differences in classification can occur depending on the guideline used.

**Table 1.1 Current COPD Airway Severity Classifications**

Classification		Lung function results		
Stage	Severity	Post bronchodilator FEV <sub>1</sub> / FVC ratio	Post bronchodilator FEV <sub>1</sub> % predicted *	FEV <sub>1</sub> % predicted #
1	Mild	< 0.7	≥ 80%	≥ 80%
2	Moderate	< 0.7	50-79%	50% ≤ FEV <sub>1</sub> < 80%
3	Severe	< 0.7	30-49%	30% ≤ FEV <sub>1</sub> < 50%
4	Very severe	< 0.7	< 30%  Or < 50% with chronic respiratory failure	< 30%  Or < 50% with chronic respiratory failure
* NICE 2010 COPD severity classification (NICE, 2010a)				
# GOLD 2013 COPD severity classification (Decramer et al., 2013)				

**Table 1.2 ATS/ERS COPD Severity Classification (ERS, 2004)**

Classification	Lung function results	
Severity	FEV <sub>1</sub> / FVC ratio	FEV <sub>1</sub> % predicted
At risk	> 0.7	≥ 80%  <b>Risk factors:</b> smoking history, exposure to pollutants <b>Symptoms:</b> cough, sputum production, dyspnoea or family history
Mild	≤ 0.7	≥ 80%
Moderate	≤ 0.7	50% ≤ FEV <sub>1</sub> < 80%
Severe	≤ 0.7	30% < FEV <sub>1</sub> < 50%
Very severe	≤ 0.7	FEV <sub>1</sub> ≤ 30%

#### *1.6.1.b Other severity measures*

It is becoming increasingly important to stage COPD severity on measures other than purely FEV<sub>1</sub> to account for the systemic effects of COPD and to better define the respiratory impairment. It is highlighted that measures such as BMI (Vestbo et al., 2006), exercise capacity (Pinto-Plata et al., 2004), exacerbations (Donaldson et al., 2002) and quality of life (Stahl et al., 2005) contribute to disease progression, thus should be taken into consideration when assessing disease severity; as well as the use of the Medical Research Council (MRC) dyspnoea scale for prognostic evaluation. Gas transfer, partial pressure of oxygen in arterial blood and cor pulmonale should then be investigated in secondary care (NICE, 2010a). This allows for a more thorough assessment of the disease severity and allows the condition to be graded appropriately. The COPD assessment test (CAT) questionnaire provides a reliable measure of COPD related health status which can be used for long term follow up of COPD patients (Jones et al., 2009).

#### *1.6.1.c Measuring airway inflammation*

Airway inflammation can be measured in a variety of ways, including the fraction of exhaled nitric oxide (FeNO), which is a repeatable measure in stable COPD subjects (Rouhos et al., 2011), though the diagnostic use is not as fully understood as it is in asthma. Increased nitric oxide measurements are well established in asthma, but it has also been suggested that FeNO may reflect neutrophilic inflammation in COPD (Silkoff et al., 2001).

Sputum cell counts can help define the inflammatory phenotype – where COPD sputum samples consist predominantly of neutrophils (Tsoumakidou et al., 2003).

Inflammatory markers in induced sputum have been shown to be stable and neutrophilic inflammation repeatable in moderate, clinically stable COPD patients (Beeh et al., 2003). There is a negative correlation between sputum neutrophils and FEV<sub>1</sub> (Peleman et al., 1999). A study by Stanescu *et al.* investigating the rate of decline of lung function with airway inflammation found that sputum with increased neutrophils correlated with the decline in FEV<sub>1</sub> in both male smokers and non-smokers over the 15 year follow-up (Stanescu et al., 1996).

#### *1.6.1.d Composite measures of disease severity*

There have been a number of composite measures explored - the BODE (BMI Obstruction Dyspnoea Exercise) index, has been shown to be highly predictive of COPD mortality and is commonly used to assess disease severity (Celli et al., 2004). It is a 10 point scale in which the higher the score the greater the risk of death. This provides a systematic grading system to reflect the composite nature of COPD (Celli et al., 2004).

The ADO (Age Dyspnoea Obstruction), a simplified scoring system which also represents the multidimensional aspects of COPD has been developed (Puhan et al., 2009), but its implementation remains to be seen. This index uses age, dyspnoea and obstruction to determine a score to aid prognostic assessment. Dyspnoea is assessed with the MRC dyspnoea scale and obstruction is measured with FEV<sub>1</sub> (Puhan et al., 2009).

## **1.7 Treatment**

### **1.7.1 Smoking Cessation**

Smoking cessation is the only current intervention able to slow the rate of lung function decline associated with COPD progression (Scanlon et al., 2000). Reluctance of people to accept smoking cessation help is often due to lack of awareness of services available to them. Smoking cessation services therefore need to be appropriately publicised and promoted, particularly in more deprived areas, with emphasis on a personalised, non-judgemental service (Roddy et al., 2006). Nicotine replacement therapy and brief counselling has been shown to be an effective smoking cessation intervention in in-patients. This intervention significantly increases the number of people who were still abstaining from smoking a year later (Molyneux et al., 2003). Other therapies such as bupropion and varenicline are also effective in aiding smoking cessation (Jorenby et al., 2006).

### **1.7.2 Pharmacology**

Current treatment for COPD concentrates on patient reported symptoms such as bronchodilators for breathlessness. Short acting  $\beta_2$  agonists and anticholinergics are routinely prescribed for symptomatic relief of breathlessness, and can be used in combination (NICE, 2010a) as they can be synergistic (Dorinsky et al., 1999). If patients remain symptomatic or have two or more exacerbations per year a long acting bronchodilator should be prescribed.

Long acting anticholinergic bronchodilators, such as Tiotropium, have been shown using results from the UPLIFT (Understanding Potential Long-term Impacts on



Function with Tiotropium) study to improve lung function, health-related quality of life (HRQoL) and exacerbations (Tashkin et al., 2010).

Inhaled corticosteroids (ICS) alone are not licensed, but they have been shown to reduce exacerbations and improve quality of life; and are licensed to be used in combination with long acting B<sub>2</sub>-agonists (Sin et al., 2003b, NICE, 2010a). ICS are recommended in those patients with an FEV<sub>1</sub> ≤ 50% predicted and having two or more exacerbations requiring oral corticosteroids or antibiotics per year (Calverley et al., 2006, NICE, 2004).

Theophyllines are not mainstay treatment but are occasionally used (NICE, 2010a). There is no place for routine oral corticosteroid maintenance therapy (Rice et al., 2000). Long term oxygen therapy has been shown to improve survival by 40% when given to patients with chronic hypoxia (Stuart-Harris et al., 1981).

During an exacerbation an increase in medication will be required, and severe exacerbations may require hospital admission. An exacerbation is defined as ‘a sustained worsening of the patient’s symptoms from his or her usual stable state that is beyond normal day-to-day variations, and is acute in onset’ (NICE, 2010a). Such exacerbations may require oral corticosteroids (OCS), nebulisers, antibiotics and/or supplemental oxygen (NICE, 2004). Hospital admissions may be due to the severity of exacerbation or because the patient is unable to cope at home.

### **1.7.3      Non-pharmacological Treatment**

Pulmonary rehabilitation should form an integral component of the management of respiratory disease, including COPD patients (Bolton et al., 2013, Spruit et al., 2013). Rehabilitation programmes include education, breathing exercises and exercise

training; and have been shown to improve exercise tolerance, QoL, dyspnoea and improve psychological well-being (Griffiths et al., 2000).

Vaccinations have been shown to reduce mortality due to influenza in COPD patients (MacNee and Calverley, 2003). Influenza vaccination is therefore recommended annually (Hak et al., 1998). Pneumococcal vaccination has been demonstrated to reduce episodes of pneumococcal pneumonia in COPD patients younger than 65 years old with severe airflow obstruction (Alfageme et al., 2006). The importance of health education programmes aimed at informing patients about their condition, and how to adapt their daily routine to deal with their disease is now recognised (Worth and Dhein, 2004). These self-management programmes require a multi-disciplinary approach (Bourbeau et al., 2004), thus there is a need to establish new approaches in COPD care.

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*COPD is a slow but continuously progressive condition, causing constantly changing symptoms. It is estimated that two-thirds of COPD subjects have at least one co-morbidity as a result of their condition (Raheison and Girodet, 2009).*

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## **1.8 Comorbidities**

The systemic consequences of COPD include cardiovascular, musculoskeletal, psychological problems such as depression and anxiety, cancer, and endocrine problems such as diabetes and metabolic syndrome (Luppi et al., 2008). It was previously thought that comorbidities only presented in the latter stages of COPD,

but recent studies have demonstrated a high proportion of patients with musculoskeletal and cardiovascular disease even in mild airway obstruction (Sabit et al., 2007, Malerba and Romanelli, 2009, Bolton et al., 2004).

#### **1.8.1      Depression and Anxiety**

Prevalence estimations for depression and anxiety vary widely (Hill et al., 2008). De Godoy *et al.* detected anxiety prevalence in COPD patients of 53% using the Beck Anxiety Inventory and depression at 46% using the Beck Depression Inventory (de Godoy and de Godoy, 2003). Depression and anxiety have been reported in patients with mild COPD and prevalence rates were higher among women (Di Marco et al., 2006).

#### **1.8.2      Lung Cancer**

Cigarette smoking is the major cause of lung cancer (Wasswa-Kintu et al., 2005). An obstructive ventilatory limitation, as demonstrated by a reduced FEV<sub>1</sub>, increases the risk of developing lung cancer. As lung function deteriorates and increases in severity, the risk of developing lung cancer also increases (Wasswa-Kintu et al., 2005). Airway inflammation could have an important role in the pathogenesis of lung cancer because it is thought chronic inflammation increases the progression of carcinogenesis (Ballaz and Mulshine, 2003), providing a possible link between COPD and lung cancer. A second plausible explanation for the relationship between reduced lung function and lung cancer is that with greater airflow obstruction people are less able to clear carcinogens from the airway, making them more at risk of developing lung cancer (Wasswa-Kintu et al., 2005).

### **1.8.3      Body Composition**

#### *1.8.3.a Cachexia*

Cachexia is defined by Kotler as the 'clinical consequence of a chronic systemic inflammatory response' resulting in loss of weight and muscle mass, due to the loss of both fat and non-fat components (Kotler, 2000), and occurs in 20-25% of COPD patients (Engelen et al., 1994, Wagner, 2008). Loss of skeletal muscle mass and function leads to reduced health status and exercise endurance, fatigue, dyspnoea and impaired cardiac function; which in turn can result in immobility and increased mortality (van Eeden and Sin, 2008). BMI is a surrogate measure and does not account for losses in specific tissue – such as fat or skeletal muscle.

Low BMI has been found to be an independent risk factor for COPD mortality, with strongest association in more severe COPD patients (Landbo et al., 1999, Schols et al., 2005). Additionally, in severe COPD patients with a normal or low BMI, weight gain seems to be beneficial (Schols et al., 1998).

There are several mechanisms to determine body composition including bioelectrical impedance analysis (BIA), dual energy x-ray absorptiometry (DEXA) and skinfold assessment. They aim to differentiate fat mass (FM) and fat-free (muscle, bone and organs) mass (FFM). DEXA has the additional benefit of measuring bone mineral density (BMD) measurement. Patients with a normal BMI can have loss of fat free mass, i.e. hidden loss (Bolton et al., 2004). This loss of FFM, irrespective of BMI, has been associated with increased morbidity, worsening quality of life (Shoup et al., 1997) and mortality (Schols et al., 2005). Indeed, COPD patients with muscle atrophy have comparable mortality risk to those with cachexia (Schols et al., 2005).

Fat free mass index (FFMI) has been reported to be a stronger predictor for mortality in COPD patients than BMI (Schols et al., 2005).

As airflow obstruction severity increases there is preferential loss of skeletal muscle mass, reflecting a continuous catabolic state (Eid et al., 2001). Originally tissue wasting in COPD was thought to be caused by only the imbalance of calorific intake and expenditure; however, it is now perceived to be due to multiple factors:-

#### *1.8.3.b Likely causes of altered body composition*

Muscle wasting occurs due to the imbalance between protein synthesis and protein breakdown. The exact nature in COPD remains to be elucidated, but it is likely that altered anabolic and catabolic mediators play their part (Schols, 2000).

Nutritionally, many patients with COPD eat sub-optimally because of the alteration in breathing pattern that occurs during chewing and swallowing, and a subsequent decrease in oxygen saturations can occur (Schols, 2000). Iatrogenic factors such as thrush, a side effect to some inhalation therapies, could also affect eating habits. Many patients with COPD also report loss of appetite and early satiety during exacerbations (Vermeeren et al., 1997). A fundamental feature of cachexia is hypermetabolism, defined as elevated resting energy expenditure (Kotler, 2000). Between 30% - 50% of patients with COPD experience tissue wasting, a likely result of hypermetabolism (Mannix et al., 1999). COPD patients become very inactive as their symptoms increase and they become limited by their breathlessness, compared to healthy subjects of similar age. It has been considered that this drastic loss of anabolic activity and deconditioning contributes to cachexia (Wagner, 2008).

Cachexia and muscle wasting are influenced by hormonal factors (van Eeden and Sin, 2008). There are many reasons for low Testosterone in patients with COPD, including hypoxic inhibition of Luteinizing Hormone (LH) (Semple et al., 1981), natural decline with age (Gray et al., 1991) and OCS therapy (Kamischke et al., 1998). There is differing evidence on the levels and impact of Insulin-like Growth Factor 1 (IGF-1) in patients with COPD. Some studies have shown an increase in IGF-1 which is thought to be due to physiological stress as a result of chronic hypoxia and bronchoconstriction (Creutzberg and Casaburi, 2003); whereas other studies have reported a decrease in IGF-1 levels (Burdet et al., 1997). Age and OCS are contributing factors leading to the down regulation of the growth hormone system (Creutzberg and Casaburi, 2003); which results in reduced stimulation for muscle growth, thus reduced muscle mass (Creutzberg and Casaburi, 2003).

Ghrelin stimulates the secretion of growth hormone, and has been shown to reduce fat utilisation through a positive energy balance (Tschop et al., 2000). Underweight (BMI < 20kg/m<sup>2</sup>) COPD patients have increased plasma ghrelin levels (Itoh et al., 2004); and it is thought to be elevated in response to the cachectic state of these patients. The role of leptin, an adipocyte derived hormone, include effects on energy balance and energy homeostasis, and the regulation of long term body composition (Kotler, 2000).

The increased systemic inflammation seen in COPD is energy intensive, requiring large amounts of essential amino acids, which drives the loss of skeletal muscle and subsequent decreased muscle mass. When the process is chronic the depletion of skeletal muscle mass contributes to increased mortality and morbidity (Kotler,

2000). Inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  are associated with increased metabolism and the subsequent increase in protein turnover, with prolonged exposure to increased TNF- $\alpha$  contributing to weight loss and malnutrition (de Godoy et al., 1996).

#### *1.8.3.a Obesity*

Obesity, as indicated by a BMI  $\geq 30$  kg/m<sup>2</sup>, is a major problem. Related to this there is an increased inflammatory state, associated with increased CRP levels in patients with COPD, suggesting adipocyte-induced systemic inflammation in COPD (Breyer et al., 2009).

Overweight patients with moderate to severe COPD had a lower mortality risk than normal weight patients, as mortality in those with severe airflow limitation increased with decreasing body weight (Wilson et al., 1989). Since then, in a study of over 2,130 patients with COPD, Landbo *et al.* have described a 'U' shaped relationship between BMI and mortality, where those with a low BMI had high mortality and those with a normal or high BMI had better survival with the mortality risk increasing again in the obese (Landbo et al., 1999), although the number in the obese group were small.

#### **1.8.4      Metabolic Syndrome and Diabetes**

Increased circulating C-reactive protein (CRP) and IL-6 levels are positively associated with metabolic syndrome in COPD patients; and Watz *et al.* reported in 175 stable outpatients with COPD GOLD stage I-IV and 30 stable patients with chronic bronchitis, nearly half (47.5%) of all subjects had metabolic syndrome (Watz et al., 2009), using the International Diabetes Federation criteria (IDF, 2006).

The risk of developing type 2 diabetes is increased in COPD patients, and has been demonstrated in female adults through an epidemiological study of 121,700 females with COPD or asthma, aged between 30 and 55 years (Rana et al., 2004). Glucose intolerance and type 2 diabetes mellitus are recognised complications to OCS treatment (Archer, 2009). In addition, a further mechanism behind this increased risk of developing diabetes is thought to be due to the increased inflammatory mediators present in subjects with COPD (Bolton et al., 2007). Further research is required in this area to investigate the association with inflammation, because glucose intolerance and diabetes is evident even in people not taking OCS (Mannino et al., 2008).

Insulin resistance has been assessed using the homeostatis model assessment (HOMA) which is the product of fasting serum insulin and fasting plasma glucose values (Bonora et al., 1998). Systemic inflammation has been associated with increased insulin resistance using HOMA index, in patients with COPD compared to healthy controls, which Bolton *et al.* related to the increased levels of TNF- $\alpha$  soluble receptor I and IL-6 in 56 non-hypoxaemic COPD patients compared to 29 controls (Bolton et al., 2007). TNF- $\alpha$  and IL-6 block the signalling through the insulin receptor, therefore inducing insulin resistance and increasing the risk of type 2 diabetes (Barnes and Celli, 2009, Spranger et al., 2003). CRP and IL-6 are increased in subjects with type 2 diabetes mellitus (Pickup and Crook, 1998) and these inflammatory markers are also increased in COPD.

Obesity, particularly abdominal obesity, in COPD patients is associated with metabolic and inflammatory abnormalities, such as increased insulin levels, raised



TNF- $\alpha$  and IL-6 plasma levels, decreased adiponectin and decreased HDL cholesterol; which commonly leave the patient susceptible to developing diabetes and cardiovascular disease (Poulain et al., 2008).

### **1.8.1      Osteoporosis**

There is wide variation in the prevalence of osteoporosis and osteopenia in COPD patients, with 68% of a COPD study population found to have either osteoporosis or osteopenia and a higher prevalence in females (Jorgensen et al., 2007). Clinically stable COPD patients starting pulmonary rehabilitation with GOLD stage I-IV disease severity reportedly had a prevalence of osteoporosis of 21% and osteopenia is 41% (Graat-Verboom et al., 2009). The different prevalence could be dependent on the way osteoporosis was diagnosed - there is an overall lack of awareness to look for osteoporosis until COPD progresses to severe disease. Osteoporosis was first reported by Shane *et al.* in pre-transplant end-stage respiratory disease patients, where OCS treated COPD patients were more severely affected by osteoporosis (Shane et al., 1996). Osteoporosis is now being recognised in even mild and moderate COPD (Bolton et al., 2004). Many of the risk factors for osteoporosis also occur in COPD, including age, inactivity, poor nutrition, low BMI, and inhaled and oral corticosteroids (Barnes and Celli, 2009). The risk of low bone mineral density and osteoporosis increases as the severity of COPD increases (Vrieze et al., 2007), with subjects from the general population and either a low BMI or frequent exacerbations having the highest risk (Sin et al., 2003a). Limitations of this study by Sin *et al.* include the results coming from NHANES and therefore the results were collected 15 years previously; and the subjects had airflow obstruction so the sample was not exclusively COPD subjects.

Osteoporosis prevalence is higher in patients with COPD compared to matched controls, even in those with newly diagnosed COPD (Soriano et al., 2005). There are increased fracture rates in people with COPD with both current and previous use of inhaled corticosteroids, and when oral corticosteroid courses are adjusted for the dose response relationship between ICS and hip fractures remains (Hubbard et al., 2002). Low bone mineral density is seen in patients with COPD independent of their respiratory medication (Sin et al., 2003a). Contributing factors for this low bone mineral density include decreased activity levels (Biskobing, 2002), low BMI (Incalzi et al., 2000), smoking history (Ward and Klesges, 2001), reduced testosterone levels (Kamischke et al., 1998), hypercapnia (Dimai et al., 2001) and systemic inflammation, particularly TNF- $\alpha$  (Bolton et al., 2004). Further studies are needed to look into the causation of these factors. Morbidity and mortality is further increased with vertebral compression fractures in COPD patients, because symptomatic compression fractures can further reduce already impaired respiratory function (Carter et al., 2008). A study of 74 females reported a 10% decrease in FVC in those with vertebral fractures (Leech et al., 1990).

### **1.8.2      Cardiovascular (CV) Disease**

#### *1.8.2.a   CV disease and lung function*

The association between lung function and cardiovascular disease has been shown in the general population. FEV<sub>1</sub> can be used as a marker of cardiovascular mortality, independent of age, gender, smoking history (Sin et al., 2005), and an independent predictor of all-cause mortality (Stavem et al., 2005). Even modest reductions in FEV<sub>1</sub> (Sin et al., 2005, Hole et al., 1996) are a significant risk factor for

cardiovascular disease. In an epidemiological sample of 621 68 year old males, Engstrom *et al.* demonstrated a high occurrence of ventricular arrhythmia, which had an inverse relationship with airflow obstruction and was associated with increased mortality and CV events (Engstrom et al., 2001). A prior population based cohort study by Engstrom *et al.* in 291 male smokers reported increased CV risk and mortality in those with a declining lung function; suggesting change in lung function may assess individual susceptibility (Engstrom et al., 2000); consistent with that of Tockman *et al.* where it was reported that in a population of white males without a history of CV disease, decline in FEV<sub>1</sub> was related to CV mortality independent of CV risk factors (Tockman et al., 1995).

#### *1.8.2.b CV disease and COPD*

The importance of investigating and treating cardiovascular disease in patients with COPD is highlighted by Mannino *et al.*, where it is reported COPD patients are more likely to die of CV complications than of respiratory failure (Mannino et al., 2006). A placebo controlled RCT comparing different inhaled medications (salmeterol, fluticasone propionate and combination) in 6184 moderate-severe patients with COPD from 42 countries reported that 26% of deaths in patients with COPD were CV in nature across the arms (McGarvey et al., 2007). In a retrospective cohort study with 11,493 COPD subjects and 22,986 controls, it was reported that patients with COPD have a significantly higher risk of congestive heart failure, arrhythmia and myocardial infarctions than the control group (Curkendall et al., 2006), and patients with COPD have a higher incidence of first time acute MI compared to the general population without COPD (Feary et al., 2010).

A population based cohort study looking at patients with COPD found statins to be protective against CV mortality, and reported statin use to be associated with improved survival irrespective of co-existent CVD (Sheng et al., 2012).

The structural and functional relationship between the respiratory and CV system may explain why COPD is a risk factor for CV diseases – as the one organ system becomes impaired, it impacts upon the function of the other; so as COPD impairs the respiratory system the CV system is negatively affected. This may be as a result of common pathologies and risk factors such as smoking, age and sedentary lifestyles or conditions that result in impairment of the heart from primary lung disease (Barnes and Celli, 2009, Maclay and MacNee, 2013).

A longitudinal epidemiological study reported that factors other than the obstructive lung disease itself, such as comorbidities including cardiac arrhythmias, were important in linking COPD to causes on the death certificate, as patients may die with, rather than of, COPD (Camilli et al., 1991).

An epidemiological study reported low grade systemic inflammation in subjects with moderate to severe airflow obstruction and associations with increased risk of cardiac injury; therefore proposed systemic inflammation as a possible explanation for the increased rates of CV complications in patients with COPD (Sin and Man, 2003, Agusti, 2005).

Smoking has a number of effects that may promote atherosclerosis through vascular inflammation and oxidative stress (Perlstein and Lee, 2006). Systemic inflammation and increased insulin resistance, both of which are present in subjects

with COPD, are key mechanisms in the development of atherosclerosis and therefore increases the prevalence of cardiovascular disease (Bolton et al., 2007, Mannino et al., 2006). It is not yet fully understood whether CV disease leads to increased inflammation, increased inflammation contributes to the development of CV disease, or if there is another factor that promotes both inflammation and CV disease in patients with COPD.

Some patients with COPD develop right-sided heart failure (RHF) as their condition progresses, due to worsening pulmonary arterial hypertension as a result of hypoxaemia; however, the incidence of true cor pulmonale appears to be declining. This may be because the clinical assessment and prescription services for supplemental oxygen therapy has improved, enabling hypoxic patients to receive oxygen therapy earlier (Barnes and Celli, 2009). Structural changes in the right ventricle may impact upon the left ventricle, and it has previously been reported that COPD patients also have left ventricular diastolic dysfunction (Funk et al., 2008, Sabit et al., 2010), and unrecognised heart failure co-exists in patients with COPD (Le Jemtel et al., 2007). One of the major determinants for left ventricular diastolic dysfunction in COPD was aortic stiffness (Sabit et al., 2010).

There is a high prevalence of myocardial injury in COPD, commonly occurring during severe acute exacerbations (Patel et al., 2013), with a recent prospective study in 242 patients with COPD reporting that one in 12 patients admitted to hospital with an acute exacerbation of COPD met the current Universal Definition for Myocardial Infarction (McAllister et al., 2012). Using an epidemiological approach it has been reported that COPD exacerbations increase the risk of MI and stroke, where acute

exacerbations of COPD are associated with a 2.27 fold increased relative risk of MI during the 5 day post exacerbation period (Donaldson et al., 2010).

Studies looking at hard CV endpoints, such as death and MI events, have found increased CV disease prevalence in patients with COPD compared to controls (Feary et al., 2010, Sin et al., 2006); however, we ideally need a non-invasive measure to detect CV risk.

#### *1.8.2.c Non-invasive measures of CV risk*

There are many methods to non-invasively measure CV risk, including arterial stiffness, carotid intima media thickness (CIMT) and endothelial function.

CIMT is the distance from lumen-intima interface to the media-adventitia interface of the artery wall, measured using ultrasonographic images of the carotid arteries (Polak et al., 2011). CIMT has been reported using subjects in the Framingham Offspring Study cohort, to be an independent predictor of CV events in subjects without a history of CV disease (Polak et al., 2011). In contrast, a meta-analysis reported that CIMT progression was not associated with the risk of subsequent CV events (Lorenz et al., 2012) and despite being predictive of CV endpoints CIMT did not improve risk classification in the general population (Lorenz et al., 2010).

Endothelial function can be measured by flow mediated dilation, is predictive of future CV events in the general population (Lind et al., 2011), and in patients with COPD was associated with severity of airflow obstruction (Clarenbach et al., 2013) and systemic inflammation (Eickhoff et al., 2008), although contentious with other methods (Maclay et al., 2009).

Coronary artery calcium scores, as determined by CT scans, are increased in patients with COPD compared to both smoker and never smoker controls, and are associated with increased mortality (Williams et al., 2014).

Carotid – femoral ‘aortic’ PWV is used as the measure of arterial stiffness because it is considered the gold standard measure of arterial stiffness and the most clinically relevant measure; due to the aorta being what the left ventricle first ‘sees’ therefore being responsible for the majority of pathophysiological effects of arterial stiffness (Laurent et al., 2006). Pulse wave velocity is the speed at which a pressure wave travels between two points, for example between the carotid and femoral pulse points; and is calculated by dividing the distance from the carotid to femoral point by the transit time – being the time of travel of the foot of the wave over the distance measured (Laurent et al., 2006). A systematic review with 17,635 participants, presenting data from 16 studies reported that aortic PWV enabled better identification of those with high CV risk who could therefore benefit from CV risk management (Ben-Shlomo et al., 2013).

Aortic PWV is an independent predictor of CV disease (Laurent et al., 2006), where a greater PWV indicates stiffer arteries (Mattace-Raso et al., 2006). Aortic stiffness has been studied as a non-invasive predictor of cardiovascular events in COPD, and has been shown to be higher in patients with COPD compared to matched smoker controls (Sabit et al., 2007, John et al., 2013).

#### *1.8.2.d Aortic stiffness and lung function*

The association of aortic stiffness to spirometric measures in COPD was demonstrated by Bolton *et al.*, where a strong inverse association in the Caerphilly

Prospective study with 800 male participants was reported (Bolton et al., 2009a). In addition, a French study of 194 males without CV disease reported a reduced lung function was independently associated with aortic stiffness (Zureik et al., 2001).

#### *1.8.2.e Aortic stiffness and COPD*

Aortic stiffness is increased in patients with COPD compared to controls (Sabit et al., 2007, Maclay et al., 2007), with arterial stiffness relating to emphysema severity (McAllister et al., 2007), severity of airflow obstruction, and systemic inflammation (Sabit et al., 2007). Arterial stiffness increases acutely during an exacerbation of COPD, with those patients who have frequent exacerbations having a higher aortic PWV compared to those with less frequent exacerbations (Patel et al., 2013).

Augmentation Index (Alx) is a measure of wave reflection, measured as the pressure difference between the second and first systolic peaks expressed as a percentage of the pulse pressure (Laurent et al., 2006). A large epidemiological cross-sectional study found no difference in Alx between COPD and controls when adjusted for CVD risk factors but an inverse relationship between Alx and FEV<sub>1</sub> and FVC was found (Janner et al., 2012). These findings are not unexpected as Alx has previously been reported as a less useful marker of arterial stiffness in the age group of patients with COPD because it plateaus with age (McEniery et al., 2005).

The clinical studies of arterial stiffness in COPD are presented in Table 1.3, comprising of cross-sectional, cohort, case control and RCT studies. The literature search was performed for English language studies. With the exception of one study, all other literature presented is from 2007 onwards.



#### *1.8.2.f Consequences and mechanisms of increased aortic stiffness*

With age there is a progressive increase in arterial stiffness, leading to increased pulse pressure (Domanski et al., 1999). Not only are large arteries a conduit, but they also play an important role in buffering the cyclic changes in blood pressure caused by intermittent ventricular ejection (Nichols and O'Rourke, 1998). With increased arterial stiffness there is early return of reflected waves during late systole (as opposed to diastole), which in turn increases central pulse pressure and therefore the load on the ventricle, reducing ejection fraction and increasing the myocardial oxygen demand (Boutouyrie et al., 2002). Increased arterial stiffness raises blood pressure and therefore cardiovascular risk, with cardiovascular morbidity and mortality being due to systolic hypertension affecting coronary perfusion (Laurent et al., 2001).

#### *1.8.2.g Potential mechanisms for increased arterial stiffness in COPD*

Recent findings using 153 COPD patients enrolled into a multicentre RCT reported that the increased aortic PWV seen in patients with moderate to severe COPD could be predicted using age, BP and thoracic aortic calcification (Bhatt et al., 2014).

Systemic inflammation and endothelial dysfunction have been proposed as potential explanations for the increased aortic stiffness and CV risk in subjects with COPD, where aortic stiffness can be affected by both structural and functional aspects of the conduit arteries and vascular beds (Mills et al., 2008).

As mentioned earlier, systemic inflammation is thought to provide a mechanistic link between COPD and its comorbidities, including CV disease (MacNee, 2013). Increased oxidative stress is thought to have a role in activating genes involved in

producing an inflammatory response (Alexander, 1995). The cyclic stress affecting arterial wall thickening is a possible reason for the correlation between increased arterial stiffness and atherosclerosis (Boutouyrie et al., 2002). Inflammation and arterial wall oxidative stress – as shown by elevated levels of macrophages and IFN- $\gamma$  secreting lymphocytes (Yan and Hansson, 2007) – are central in the pathogenesis of atherosclerosis (Alexander, 1995) and comparable to the inflammation seen in the peripheral lung of COPD patients (Yan and Hansson, 2007). In the presence of hyperlipidemia this inflammatory response may lead to the formation of atherosclerotic plaques (Alexander, 1995).

**Table 1.3 Arterial Stiffness and COPD Literature Summary**

Authors	Year	Study Design	Comparator	Significant exclusions	Sample size	Primary Variable	Secondary Variables	Device	Primary Outcome	Secondary Outcome	Conclusion
Bartels (Bartels et al., 2004)	2004	Single blinded, crossover	Suppl. O <sub>2</sub> v. CA	Abnormal ECG	70 COPD	Alx, BRS		T	BRS greater following suppl. O <sub>2</sub> compared to CA	Alx was increased with suppl. O <sub>2</sub> compared to CA	Suppl. O <sub>2</sub> improves BRS
Sabit (Sabit et al., 2007) *(Sabit et al., 2010)	2007	Cross-sectional	Healthy current or ex- smokers	LTOT, OCS, CVD, malignancy, cor pulmonale, inflammatory or metabolic condition, DM	75 COPD 42 controls	aPWV	BP, Alx, BMD, IL-6, TNF- $\alpha$ ,	S	Increased aPWV in COPD compared to controls, COPD:11.4 (2.7) m/s to 9.0 (1.7)m/s	Relationship between AS and airway obstruction. aPWV related to BMD	Increased AS compared to controls over a range of COPD severities; greatest in those with osteoporosis. Relates to diastolic dysfunction
McAllister (McAllister et al., 2007)	2007	Cohort study	None	Anti-inflammatories, OCS, systemic inflammatory disease, recent exac.	157 COPD	c-rPWV	HRCT	S	c-rPWV 8.7 (1.5)m/s related to emphysema severity	c-rPWV higher in GOLD stage 3-4 than 1-2	Emphysema severity is associated with AS in COPD
Mills (Mills et al., 2008)	2008	Prospective case control	Healthy controls	Recent exac, ORC, RA, IBS, OCS, connective tissue disorder, malignancy, theophylline	102 COPD 103 controls	Time to reflect (ms)	BP, Alx	S	Reduced time to wave reflection 131(1)ms in COPD	Elevated Alx in COPD	Increased AS and BP in COPD compared to controls

Maclay (Maclay et al., 2009)	2009	Case control	Healthy males	ORC, vascular function conditions or medications, inflammatory conditions	18 COPD 17 controls	aPWV	AP, Alx, VOP	S	aPWV increased in COPD 11 (2)m/s compared to controls 9 (2)m/s	Alx increased in COPD	Increased aPWV in COPD independent of smoke exposure
Vivodtzev (Vivodtzev et al., 2010)	2010	Case control Intervention in COPD	Untrained COPD and trained COPD	Recent exac., DM	10 trained 7 untrained	Change in c-rPWV	6MWD, quadriceps muscle endurance	C	c-rPWV dropped in trained patients by 1.1 (0.6)m/s from 10.3(0.7) to 9.2(0.8)m/s	Trained patients had significant improvements in 6MWD and muscle endurance	AS improves after exercise training in proportion to changes in exercise capacity
Dransfield (Dransfield et al., 2011) *(Bhatt et al., 2014)	2011	RCT of fluticasone propionate/ salmeterol	All COPD Active v. Placebo	ICS, ICS/LABA,	123 active 126 placebo	Change in aPWV	Alx	S	aPWV decreases post 12 weeks intervention with FSC compared to placebo, but did not reach significance.	Significant change in aPWV in active compared to placebo post-treatment in highest tertile (aPWV >10.9m/s). No change in Alx	FSC may reduce aPWV in those with the greatest abnormality in AS
Janner (Janner et al., 2012)	2011	Population, cross-sectional	None	Poor quality Alx, missing spirometry data	3374 general population including 494 COPD	Alx	BP	S	Alx higher in COPD than controls	Alx not increased when CVD risk adjusted for	Alx and COPD are only weakly associated

Gale(Gale et al., 2011)	2011	Prospective cohort study Pre- and post- rehab	Healthy controls at baseline only	Recent exac., IHD, cardiac failure, DM, malignancy, inflammatory or metabolic conditions, OCS	32 COPD 20 controls (baseline only)	Change in aPWV with rehab	c-rPWV, MAP, PP, Alx	S	COPD had higher PWV than controls. Rehab lowered aPWV from 9.8 (3.0)m/s to 9.3 (2.7)m/s in COPD	No change in c-rPWV or Alx following pulmonary rehab, but decreased central MAP by 8mmHg	AS improved following pulmonary rehab - likely secondary to change in MAP
Albu (Albu et al., 2011)	2011	Cross-sectional	Controls	Infection, CVD	38 COPD 36 controls	Beta stiffness index, Carotid PWV	AC, Alx	Ultra sound	COPD had higher carotid PWV than controls	AS more important in severe COPD than in mild-moderate disease	Patients with COPD have increased carotid AS independent of smoking history, and more pronounced in severe disease
Maclay (Maclay et al., 2012)	2012	Cross-sectional	Male ex-smokers	Vascular disease, systemic inflammatory conditions, statins, OCS, LTOT	16 COPD 15 controls	Skin biopsy	aPWV	S	Patients with COPD have increased skin elastin degradation compared to controls	Cutaneous elastin degradation was related to both emphysema severity and AS.	Systemic elastin degradation may be a mechanism for pulmonary, vascular and cutaneous features of COPD

Vanfleteren (Vanfleteren et al., 2013)	2013	Prospective cohort study pre- and post- rehab	Healthy controls at baseline	Unsuccessful aPWV measurements	162 COPD 168 controls	Change in aPWV	Alx	S	aPWV increased in COPD 10.7(2.7) m/s compared to controls. aPWV did not change post rehab	Alx was higher in COPD than controls	Increased AS in COPD does not respond to rehab. No change in MAP
Romme(Romme et al., 2013)	2013	Cohort	None	Anti-inflammatories, OCS, systemic inflammatory disease, recent exac.	119 COPD	c-rPWV	CAC, TAC	S	c-rPWV 8.9(1.6)m/s	Increased CAC was associated with increased AS and lower BMD	Increase in CAC in COPD associated with higher AS and lower BMD
Patel (Patel et al., 2013)	2013	Prospective cohort study	Stable v. exac.	ORC	98 Stable COPD  55 COPD with exac.	aPWV	MAP, Sputum	V	AS related to exac freq in stable state. Frequent exac 11.8 (2.1)m/s. Non frequent exacerbators 10.3 (2.0)m/s. Increase in AS with exac.	AS in stable state is reliable over time	Frequent COPD exacerbators have greater AS
Stickland (Stickland et al., 2013)	2013	Cross-sectional	Healthy controls	LTOT, DM, CVD, vasoactive medication, BMI≥32, inflammatory disorders, recent rehab, OSA	33 COPD  10 controls	c-rPWV	VO <sub>2</sub> max	T	AS increased in COPD. Physical activity and exercise tolerance is related to c-rPWV in COPD		Physical activity and exercise tolerance is significantly related to PWV in COPD

John (John et al., 2013)	2013	Cross-sectional	Healthy controls	Alpha-1 antitrypsin deficiency, malignancy, terminal disease	52 COPD 34 controls	UACR in relation to aPWV	Alx	V	aPWV related to glomerular function	aPWV greater in those with micro-albuminuria	Glomerular damage in COPD shown by increased UACR related to aPWV
Stone (Stone et al., 2013)	2013	Prospective	-	Recent exac., LTOT	23 vicorder reproducibility 33 vicorder comparison study	aPWV	Alx	V, S	Vicorder is reproducible for aPWV	Vicorder values lower, but good agreement with sphygmocor. Sphygmocor aPWV 9.5 (1.5)m/s	aPWV in COPD is reproducible and not affected by lung hyper-inflation
Cinarka (Cinarka et al., 2013)	2013	Prospective observation	Healthy controls	CVD, hypertension, DM	62 COPD 22 controls	aPWV	ABG, 6MWD	S	aPWV level is increased in COPD compared to controls	aPWV is higher in severe disease compared to mild disease	aPWV is increased in COPD, and higher in advanced compared to mild-moderate disease
Vivodtzev (Vivodtzev et al., 2013)	2013	Letter to Editor	-	-	-	aPWV	Metabolic and inflam. markers	C	Baseline aPWV 11.1 (1.9)m/s	79% of patients had abnormal aPWV >9.3m/s. Good reproducibility of aPWV.	aPWV is a reproducible measure for short to middle term intervention in COPD

Ives (Ives et al., 2013)	2013	Double blind RCT of antioxidant cocktail (vitamin C, E and $\alpha$ -lipoic acid)	Placebo	Current smokers, recent exac.	30 COPD 30 controls	FMD	c-rPWV	T	Patients FMD improved with antioxidant cocktail but not controls.	Antioxidant cocktail improved c-rPWV in patients and not controls	Patients with COPD exhibit vascular dysfunction which can be acutely mitigated by oral antioxidant
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**Abbreviations** – **aPWV**: aortic PWV, **ABG**: arterial blood gas, **AC**: arterial compliance, **Aix**: augmentation index, **AP**: augmentation pressure, **AS**: arterial stiffness, **BP**: blood pressure, **BMD**: bone mineral density, **BRS**: baroreflex sensitivity, **c-rPWV**: carotid-radial PWV, **CA**: compressed air, **CAC**: coronary artery calcification, **CVD**: cardiovascular disease, **DM**: diabetes mellitus, **exac.**: exacerbation, **FMD**: flow-mediated dilation, **FSC**: fluticasone propionate/salmeterol, **IBS**: inflammatory bowel disease, **IHD**: ischaemic heart disease, **LTOT**: long term oxygen therapy, **MAP**: mean arterial pressure, **OCS**: oral corticosteroids, **ORC**: other respiratory condition, **OSA**: obstructive sleep apnoea, **RA**: rheumatoid arthritis, **rehab**: rehabilitation, **suppl. O<sub>2</sub>**: supplementary oxygen, **TAC**: thoracic aortic calcification, **VO<sub>2</sub>max**: maximal oxygen consumption, **VOP**: venous occlusion plethysmography, **6MWD**: 6 minute walking distance

**Device Key** – S: sphygmocor, V: vicorder, C: complior, T: other tonometer device

\* A further study was published using a subset of subjects

All studies comprise Human subjects, are original studies and published in English Language.



### *1.8.2.h Inflammation and CV disease*

Many secondary comorbidities are a result of chronic systemic inflammation and account for a large proportion of the morbidity and mortality in COPD patients (Sin et al., 2006). CRP is a robust marker of the acute phase response of the innate immune response and is associated with CV disease (van Eeden and Sin, 2008). CRP impacts on the initiation, progression and stability of vascular wall atheroma through the recruitment of circulating leucocytes into blood vessel walls and macrophages uptake of low-density lipoprotein cholesterol (Torres and Ridker, 2003).

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*There is a clear need to address these co-morbidities – to improve the understanding of their causation, identifying their presence even in mild disease, and develop research into therapeutic interventions that might improve morbidity and mortality.*

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### **1.8.3      Statins**

3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors, more commonly referred to as statins, are routinely prescribed for hypercholesterolemia and cardiovascular disease and have been shown in both primary and secondary prevention to reduce cardiovascular mortality and morbidity; however there is now research to highlight their pleiotropic benefits, as highlighted in a systematic review which presented data from nine studies looking at the potential benefits of statin therapy in patients with COPD (Janda et al., 2009), and included retrospective

cohorts (Blamoun et al., 2008, Keddissi et al., 2007), a case control (Frost et al., 2007) and a RCT (Lee et al., 2008). Statins have been shown to have anti-inflammatory, antioxidant, anti-thrombogenic and have vascular function restoring actions, and immune modulating effects; as well as cholesterol lowering properties (Davignon and Leiter, 2005). Elevated high density lipoprotein (HDL) cholesterol levels have been reported in patients with severe COPD, with some of this elevation attributed to the use of Prednisolone (Reed et al., 2011).

#### *1.8.3.a Current use of statins*

Statins are currently licensed for the use of lipid lowering and subsequent cardiac prevention in subjects with hypercholesterolaemia, CV disease and high risk groups such as those with diabetes; and for primary prevention of CV disease in high risk populations (NICE, 2010b).

The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) was a randomised, double-blind, placebo controlled, parallel group, primary prevention study, which took place at 1315 sites over 26 countries. From that study it was reported that in healthy people with normal lipid levels but increased C-reactive protein (CRP) levels, atorvastatin significantly reduced CRP and importantly the incidence of CV events (Ridker et al., 2008).

The long term effects of statin therapy has been shown to be cardio-protective in a high risk population in a study with a 10 year follow up following an initial study with pravastatin. The West of Scotland Coronary Prevention Study (WOSCOPS) was a randomised, placebo controlled trial looking at the use of 40mg pravastatin in

hypercholesterolemic men without evidence of myocardial infarction. During a follow up study looking at participants 10 years after the end of the trial there was still evidence of reduced risk of major coronary events, thought to be due to the stabilisation of plaques (Ford et al., 2007).

#### 1.8.3.a.i Diabetes

The 'Collaborative Atorvastatin Diabetes Study' (CARDS) was a multi-centred placebo controlled RCT in the UK and Ireland, consisting of 2,838 patients with diabetes without CV disease. It was reported that Atorvastatin 10mg reduced the risk of first CV events by 37% and all-cause mortality by 27% in this group of patients with type 2 diabetes (Colhoun et al., 2004).

#### 1.8.3.a.ii CV disease

The Scandinavian Simvastatin Survival Study (4S) was a double blinded RCT in 4,444 patients with a history of CV disease. Over the 5.4 year median follow-up, simvastatin was shown to reduce morbidity – with a risk reduction of 30%, attributable to a 42% reduction in CV death. A reduced risk of CV events was also demonstrated in the active group compared to the placebo group. Simvastatin was reported to be beneficial irrespective of previous MI, smoking history and hypertension (Kjekshus and Pedersen, 1995).

A prospective, randomised, single-blind, single-centre study in 93 patients with Coronary Artery Disease (CAD) found that fluvastatin had a significant beneficial effect on the vascular system after 3 months of treatment; however, arterial stiffness was measured using brachial-ankle PWV (baPWV) which does not have the same predictive power as aortic PWV (Hongo et al., 2008).

The development of aneurysms is associated with a chronic inflammatory response and the increased production of matrix metalloproteinases (MMPs) (Wilson et al., 2005). The combination of ezetimibe and simvastatin in patients with abdominal aortic aneurysms (AAA) has shown a beneficial effect on the inflammatory and proteolytic components involved in aneurysm enlargement, with significant reductions in aortic wall levels of MMP-9 and IL-6 (Dawson et al., 2011). A recent meta-analysis looking at the effects of statin therapy in patients with AAA suggests that statin therapy does not affect AAA enlargement, but mortality rates following elective AAA repair are significantly improved with statin therapy (Twine and Williams, 2011).

#### *1.8.3.b Inflammation and statins*

Animal studies in rats have demonstrated statins' ability to inhibit leukocyte-endothelial cell adhesion (Kimura et al., 1997) and in in vitro studies cellular proliferation (Rudich et al., 1998), reduce oxidative stress, reduce the numbers of neutrophils, IL-6 and TNF- $\alpha$ , decrease T-cell activation and differentiation, and increase apoptosis of eosinophils using human cells in vitro. This may be of benefit to COPD patients as cigarette smoking has been shown in in vitro studies to enhance the activation of inflammatory cells including neutrophils (Inoue et al., 2000).

A double blinded RCT in 125 patients with stable COPD using pravastatin 40mg od for 6 months reported an increase in exercise time and a decrease in hs-CRP, with a correlation between the decrease in hs-CRP and increased exercise time (Lee et al., 2008). Statins have also been shown to reduce inflammation in other chronic

inflammatory conditions (McCarey et al., 2004). Airway inflammation is discussed below.

#### *1.8.3.c Statins and aortic stiffness in other disease groups*

Previous studies have also looked at the effects of statin therapy in patients with rheumatoid arthritis – another chronic inflammatory condition. Maki-Petaja *et al.* reported a decrease in aortic PWV, inflammatory markers and disease activity in patients with rheumatoid arthritis following simvastatin therapy. The comparator was ezetimibe which showed a similar change. The study design did not allow for a placebo comparator group (Maki-Petaja et al., 2007).

It has been suggested that statin therapy in COPD could modify non-invasive markers of cardiovascular disease, such as arterial stiffness, in the short term. Pilot data looking at sub-therapeutic, low-dose fluvastatin on arterial wall properties has shown a decrease in PWV at both 14 and 30 days after starting 10mg fluvastatin therapy in a double-blind randomised trial with 50 healthy male subjects. The greatest improvement in PWV was seen after 30 days of treatment in apparently healthy males, with a non-significant improvement maintained after 5 months (Lunder et al., 2011).

#### *1.8.3.d Statins and COPD/ Airflow obstruction*

There is limited but supportive evidence for a cardioprotective role for statins in COPD: retrospective, observational studies suggest statins confer benefit. A time-matched nested case-control study of two population-based retrospective cohorts by Mancini *et al.* reported that statin treatment in patients with COPD may be cardio-protective and therefore modify disease prognosis (Mancini et al., 2006).

More recently a prospective observational study has reported that long term statin use decreases all-cause mortality by 39% in patients with COPD, whilst short term statin use reduces respiratory mortality by 64% (Lahousse et al., 2013). Reduced mortality and risk of death from influenza/pneumonia has also been reported following statin treatment in patients with COPD (Frost et al., 2007).

Studies using atorvastatin have also shown a significant reduction in deaths as a result of infection and respiratory illness and therefore a reduction in all-cause mortality as a result of decreased non-CV deaths (Sever et al., 2011).

Studies looking at the use of statins in asthma have failed to show any improvement in physiological parameters or sputum eosinophils (Menzies et al., 2007), but a recent RCT by Hothersall *et al.* in 54 mild to moderate atopic asthmatics has reported a decrease in sputum macrophages when atorvastatin was used with inhaled corticosteroids over 8 weeks, despite no improvement in lung function or FeNO, which also encourages there may be a potential role for statins in airways diseases in which activated alveolar macrophages are involved in the pathogenesis, such as COPD (Hothersall et al., 2008).

The potential benefit of these pleiotropic effects of statin therapy in current and ex-smokers has been suggested in a retrospective study by Keddissi *et al.* to be associated with a reduced rate of decline in lung function, predominantly spirometry measurements, and a lower incidence of respiratory related emergency hospital admissions, regardless of the underlying lung pathology in a study comparing current and ex-smokers to a control group (Keddissi et al., 2007). In 2007 Alexeeff *et al.* reported a reduction in the lung function decline in people

concurrently taking statin therapy, based on a population of 803 older males from the Veterans Administration Normative Aging Study, with both smoking and non-smoking backgrounds. Approximately a third of subjects in the statins group were never smokers and there was a pack year history in the statin and not on statin groups of 31 and 29 years respectively (Alexeeff et al., 2007). More recently, epidemiologically the presence of airflow limitation was found to be 5 times lower in those taking statins compared to people not on statin therapy (Bando et al., 2012).

The potential beneficial effects of statin therapy on lung function may be due to their anti-inflammatory properties. The systemic inflammatory mediator CRP has been associated with an increased risk of CV disease (Ridker, 2003), inversely associated with lung function in people even without respiratory disease (Aronson et al., 2006), and elevated in elderly subjects with obstructive lung disease compared to age matched controls (Yende et al., 2006), and in COPD populations (Pinto-Plata et al., 2006). Both airway and systemic inflammatory state are thought to increase over time in COPD patients, and this heightened inflammatory state contributes to the decline in lung function (Donaldson et al., 2005).

No difference in the rate of decline in FEV<sub>1</sub> or FVC was seen between different types of statins (Keddissi et al., 2007), however lipophilic statins, such as simvastatin and atorvastatin, are thought to have the greatest anti-inflammatory potential (Kiener et al., 2001). The urgent requirement for prospective drug trials in order to appropriately assess the impact of statins on clinically relevant outcomes in COPD patients has been highlighted (Janda et al., 2009).

Studies looking at the use of statins during exacerbations of COPD have been reported. A prospective longitudinal evaluation of statin use on the outcomes of 245 patients admitted to hospital with COPD exacerbations has shown that patients on statin therapy presented a lower total number of exacerbations during the 1 year follow-up, and also had a longer period before the following exacerbation. There was also an improvement in HRQoL post exacerbation hospital discharge in those patients on statin therapy, which could be attributable to the reduction in exacerbations and longer duration between exacerbations. There was no short or long term survival improvements after hospitalisations with COPD exacerbations (Bartziokas et al., 2011). A more recent observational study supports these findings in suggesting statin use reduces the risk of COPD exacerbations (Wang et al., 2013).

In a retrospective study the use of statins were associated with improved survival after exacerbations of COPD. The use of ICS further increased the survival benefit associated with statin treatment (Soyseth et al., 2007).

Taken together there are a number of studies looking at the use of statins in patients with COPD and airflow obstruction through retrospective, population based and RCT studies, as summarised in Table 1.4. Human studies presented were published in English between 2006 and 2013.

#### 1.8.3.d.i Animal studies

Wright *et al.* has shown how statins can reverse pulmonary hypertension, which can be a serious complication of COPD, and vascular remodelling in female guinea pigs. Smoke induced endothelial dysfunction can also be significantly reversed



using statins along with the prevention of emphysema development; but there was no protective effect against small airway remodelling (Wright et al., 2010).

In a study where rats were exposed to cigarette smoke for 16 weeks, simvastatin was shown to ameliorate the structural and functional damage to the lungs, specifically, lung parenchymal destruction was attenuated, through anti-inflammatory effects and MMP-9 induction suppression (Lee et al., 2005). Consistent with in vitro studies showing statins effect in reducing inflammation, namely CRP (Kleemann et al., 2004) using male human CRP transgenic mice, unrelated to lipid lowering.

**Table 1.4 Statins and COPD/ Airflow Obstruction Literature Summary**

Authors	Year	Study Design	Drug Dose	Duration	Significant exclusions	Sample size	Primary Outcome	Secondary Outcome	Conclusion
<b>COPD</b>									
Mancini (Mancini et al., 2006)	2006	Retrospective cohort (nested case control) Quebec region	Variable	Not known	<65yrs at time of surgery, MI in previous 5 years	946 cases 18774 controls	Hospitalisation	Death	Statins were associated with reduced COPD hospitalisation and death irrespective of CV risk profile
Melbye (Melbye et al., 2007)	2007	Population based study	Variable	Not known	None reported	535 exposed 3342 unexposed	CRP level		No association between statin use and CRP levels in COPD without CVD
Soyseth (Soyseth et al., 2007)	2007	Retrospective cohort	Variable	Not known	None reported	118 exposed 736 unexposed	All- cause mortality		Statin use was associated with improved survival after COPD exacerbation
Frost (Frost et al., 2007)	2007	Retrospective cohort	Variable	≥90 days	None reported	7475 exposed to <4mg/d 11583 expose to >4mg/d 54174 unexposed	Mortality		Reduced risk of mortality from COPD in statin users that was more pronounced with moderate dose statins than low dose statins
Ishida (Ishida et al., 2007)	2007	Population based analysis	Variable	-	None reported	47 prefectures of Japan	COPD mortality	Senility Pneumonia Accidents All-cause mortality	Statin use was associated with decreased mortality from COPD

Keddissi (Keddissi et al., 2007)	2007	Retrospective cohort	Not stated	≥3 months	Normal spirometry, asthma, non-smokers	215 exposed 203 unexposed	Respiratory related emergency department visits	Decline in FEV <sub>1</sub> Decline in FVC	Statins were associated with a slower decline in lung function and reduced respiratory related emergency visits
Blamoun (Blamoun et al., 2008)	2008	Retrospective cohort	Variable	≥1 year	Incorrect COPD diagnosis, statins <1year, heart failure, pneumonia, lung surgery, OCS, FEV <sub>1</sub> <20% predicted	90 exposed 95 unexposed	Exacerbations	Intubations Morbidity	Statins may be associated with lower incidence of exacerbations and intubation in COPD
Lee (Lee et al., 2008)	2008	RCT	Pravastatin 40mg	6 months	Allergic rhinitis, wheeze, PE, reversibility	62 active 63 placebo	Increase in exercise time on treadmill	Change in CRP levels	The active group had increased exercise capacity and decrease in CRP
Van Gestel (van Gestel et al., 2008)	2008	Retrospective cohort	Variable			330 statin 980 non statin	Short term mortality (30 days)	Long term mortality (10 years)	Statin use was associated with improved short and long-term survival in patients with PAD and COPD
Bartziokas (Bartziokas et al.)	2011	Prospective	Not stated	>30days	Other respiratory condition, no spirometry data	74 statins 171 non statins	All-cause mortality within 30days of admission and in 1year follow up	Composite adverse outcome index. Number of ECOPD. Time to first subsequent ECOPD. Hospitalisations for ECOPD.	Statins were associated with lower risk of subsequent ECOPD and severe ECOPD, and improved QoL in patients hospitalised for ECOPD

								Time to first subsequent hospitalisation	
Sheng (Sheng et al., 2012)	2012	Cohort study	Not stated	-	-	PP:617 statin 657 non statin SP:292 statin 151 non statin	Total cholesterol change from baseline, CV events	All-cause mortality	Statins were protective from CV events and mortality in SP; and improved all-cause mortality in PP and SP
Lahousse (Lahousse et al., 2013)	2013	Nested case control	Variable	17 year follow up	Asthma	363 cases 2345 controls	All- cause mortality	Hs-CRP	Statin use is associated with a beneficial effect on all-cause mortality, depending on baseline systemic inflammation
<b>ASTHMA</b>									
Menzies (Menzies et al., 2007)	2007	Double-blind crossover RCT	Simvastatin 20mg/40mg	1 month	Smokers, statins, recent ECOPD	26	FeNO	Methacholine challenge, sputum cell counts, spirometry, plethysography, CRP	Simvastatin did not show anti-inflammatory activity in asthmatics
Hothersall (Hothersall et al., 2008)	2008	Double- blind, crossover RCT	Atorvastatin 40mg	8 weeks	Recent ECOPD, atopy, smoker, statins	54	Lung function, sputum cell counts	Diary data, exhaled nitric oxide, mediator levels	No improvement in lung function or other markers of asthma control. Reduction in sputum macrophages.
Braganza (Braganza et al.)	2011	Double-blind parallel group RCT	Atorvastatin 40mg	4 weeks	Statins, drugs that interfere with statins	36 active 32 placebo	PEF	QoL score	Atorvastatin did not change lung function in asthmatics

Moini (Moini et al., 2012)	2012	Double-blind RCT	Atorvastatin 40mg	8 weeks	Statins, smoking, hepatitis, myopathy, recent ECOPD requiring hospitalisation	31 active 31 placebo	ACT	Lung function Eosinophil count	Atorvastatin not effective in mild-moderate asthma
Tse (Tse et al., 2013)	2013	Cohort study	Variable	24 months	<31yrs age, OCS, anti-IgE therapy, missing covariates for propensity score calculation, <36 months enrolment, baseline asthma therapy other than with concomitant ICS	14566 statins 58135 non statins	ICS dispensing	Asthma related ED visits	Statin exposure was associated with decreased OCS dispensing and asthma-related ED visits

**Abbreviations** – **ACT**: asthma control test, **CVD**: cardiovascular disease, **ECOPD**: exacerbation of COPD, **ED**: emergency department, **FeNO**: fraction of exhaled nitric oxide, **hs-CRP**: highly sensitive C-reactive protein, **OCS**: oral corticosteroids, **PAD**: peripheral artery disease, **PE**: pulmonary embolism, **PEF**: peak expiratory flow, **PP**: primary prevention, **QoL**: quality of life, **RCT**: randomised control trial, **SP**: secondary prevention

All studies comprise Human subjects, are original studies and published in English Language.

To date, the only published randomised control trial (RCT) looking at aortic PWV as a primary endpoint in patients with COPD is a double blind, placebo controlled RCT looking at the effect of fluticasone propionate/salmeterol combination inhaler, where no significant change in aortic PWV post treatment was seen, however, a sub-group analysis looking at those in the highest baseline aortic PWV tertile did find a decrease in aortic PWV following treatment (Dransfield et al., 2011).

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The introduction highlights important gaps in our understanding. There is a huge importance of CV disease in COPD, and yet we do not currently perform any routine assessment of CV disease at either diagnosis or annual follow-up. Further, we have no COPD management that allows for primary prevention of CV disease.

In my thesis, I will present 2 studies that will contribute to our understanding of these 2 aspects, exploring potential new biomarkers and primary prevention therapy.

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# **Chapter 2**

## **Methods**

## **2 Methods**

This thesis comprises two core studies – ‘The Cardiovascular and Inflammatory Effects of Statin Therapy in COPD’ and ‘The Association of Lung Function and Cardiovascular Risk’. This methods section largely relates to the statin RCT, but many of the procedures are present in both studies, and are cross-referenced accordingly.

### **2.1 The Cardiovascular and Inflammatory Effects of Statin Therapy in COPD: Trial design**

This study was a randomised, double blind, placebo controlled, parallel group clinical trial performed at a single centre. The objective of the study was to assess whether six weeks therapy with simvastatin (20mg) od improves the increased arterial stiffness and inflammation seen in COPD patients.

The primary outcome measure was the difference in change in arterial stiffness as measured by aortic pulse wave velocity (PWV) after six weeks of treatment between the active statin treatment and placebo group.

The secondary outcome measures included the change in circulating inflammatory mediators, exhaled nitric oxide, induced sputum cell counts, exercise tolerance and lung function after six weeks treatment with either the active simvastatin or placebo.

The study was registered on the clinicaltrials.gov website – ref: NCT01151306.



### **2.1.1      Approvals**

In order to conduct this study approval had to be granted by the National Research Ethics Service (NRES), the Medicines and Healthcare products Regulatory Agency (MHRA) and Research and Development (R&D). Approvals were applied for through the Integrated Research Application System (IRAS).

#### *2.1.1.a Ethics*

All documents used in the study i.e. protocol, patient information sheet - see appendix 1 p222, consent form – see appendix 2 p234, poster advert – see appendix 3 p235, questionnaires – see appendices 8-10 p249-256 and General Practitioner (GP) letters (not shown here) were approved by the regional ethics committee (REC). REC reference: 10/H0408/10. As part of the procedure, there was a committee meeting on 22<sup>nd</sup> February 2010 to discuss the study. Committee members included both medical, such as consultant physicians, pharmacists, nurses, co-ordinators, and lay people. The study was given favourable ethical opinion on 20<sup>th</sup> April 2010 following some minor changes and the submission of an external review. A substantial amendment to invite patients from primary care sites across Derby City, Derby County, Nottingham City and Nottingham County former Primary Care Trusts was made and accepted in December 2010.

#### *2.1.1.b MHRA*

The MHRA accepted the study on 12<sup>th</sup> March 2010. Documents reviewed by the MHRA were the consent form, patient information sheet, protocol, summary of product characteristics (SmPC), and the drug bottle label. Eudra CT 2009-017689-22, and the local University of Nottingham Sponsor's Protocol Code Number 09105.

Annual Development Safety Update Reports were completed as per MHRA requirements.

#### *2.1.1.c R&D*

R&D approval was granted by Nottingham University Hospitals NHS Trust on 6<sup>th</sup> May 2010 following their review of the protocol, patient information sheet (PIS), consent form, GP letter, questionnaires and poster advert. Primary care R&D approval for the substantial amendment to allow primary care recruitment was received in December 2010.

#### **2.1.1      Sample Size**

The sample size required to detect a 10% (-1m/s) reduction in aortic PWV with intervention, a significance of 0.05 and power of 0.8 was 29 patients in each arm – active and placebo treatment. A sample size of 35 in each arm was therefore being implemented to allow a sample size of 29 fully evaluable subjects to be attained after sample attrition.

Our power calculation was based on a 1m/s drop in aortic PWV as this would be considered a significant decrease in aortic PWV. An increase in aortic PWV of 1m/s has been associated with an increase in CV events by 14%, CV mortality by 15% and all-cause mortality by 15% (Vlachopoulos et al., 2010). More recently, a meta-analysis of 13 studies comprising populations of both known CV disease and population based studies, reported a change of 1m/s in aortic PWV was associated with a hazards ratio for CV events of 1.07 for a 60 year old non-smoking male without traditional CV risk factors (Ben-Shlomo et al., 2013).

### **2.1.2      Inclusion and Exclusion Criteria**

#### **Inclusion criteria:**

- Current or ex-smoker with an appropriate smoking history
- Aged between 45 and 80 years of age with confirmed COPD: FEV<sub>1</sub> 30-80% predicted, FEV<sub>1</sub>/FVC<0.7
- Salbutamol reversibility <12% or 200mls
- Able to attend regular clinic appointments and comply with requirements of protocol (in the opinion of the investigator)
- Provide written informed consent

**Exclusion criteria:**

- Currently taking statins or fibrates,
- Known hypersensitivity / side effects to statin therapy,
- Hypercholesterolaemia ( $\geq 6.5$  mmol/L),
- Significant hypoxia ( $\text{PaO}_2 < 7.3 \text{ kPa}$ ),
- Lactose intolerance,
- Alcohol excess ( $> 21$  units per week for males or 14 units for females),
- Clinically significant liver function abnormality,
- Exacerbation in the last 4 weeks,
- Condition causing the study to be detrimental to the patient,
- Co-existing condition: Rheumatoid/collagen vascular disease, diabetes mellitus, untreated hypothyroidism, inflammatory bowel disease,
- Other respiratory disease,
- Malignancy,
- History of IHD/cor pulmonale/ congestive heart failure,
- Known  $\alpha_1$  anti-trypsin deficiency,
- Elective surgery - recent, or during study period,
- Therapies including: oral prednisolone for more than 1 week in the last 6 months, disease modifying drugs e.g. gold/sulphasalazine, investigational drugs within 4 weeks of baseline, warfarin, cyclosporine, potent CYP3A4 inhibitors, grapefruit juice
- Pregnancy/ breast feeding/at risk of becoming pregnant (not using a medically acceptable form of contraception)

### **2.1.3      Recruitment**

Participants were recruited from both primary and secondary care as well as the Nottingham Respiratory Research Unit (NRRU) database.

**NRRU Database:** The database had 33 subjects when the study first began. There has been a steady increase, mainly due to ability to liaise with GP surgeries, recruit from outpatient clinics and NRRU staff attended pulmonary rehabilitation sessions; now there are 448 participants on the COPD database.

**Secondary Care:** Patients were recruited from the out-patient clinics of Dr C Bolton by personal request as well as posters placed in the out-patient waiting room. In addition, posters were placed in the lung function department, the respiratory wards and notice boards around City Hospital, Nottingham. Other respiratory physicians across the Trust were encouraged to inform us if there was a willing participant from an outpatient COPD clinic. NRRU research staff attended Pulmonary Rehabilitation groups and Breathe Easy Meetings in a recruitment effort in general for the database.

**Primary Care:** In December 2010, ethical and R&D permission were gained to liaise with GP surgeries as Patient Identification Centres (PICS). Initially 77 surgeries were approached, with 29 positively responding and 18 able to perform searches of COPD patients. Letters were sent out by the practices to the patients meeting an abbreviated inclusion / exclusion list, dependent on the surgeries' software and search capability but broadly encompassing "age", "diagnosis of COPD", "not on a statin", "not on warfarin", "no current history of malignancy". Table 2.1 lists the search where the 'response' column indicates how many patients contacted the

research department after being sent an invitation letter by their GP. In March 2012 we applied for Primary Care Research Network (PCRN) support to aid recruitment from primary care, the study having become adopted; see Table 2.2. Figure 2.1 demonstrates the accrual for the statin RCT.

Posters were also sent to some of these and other surgeries across Nottingham City and County PCT's who were willing to put them up, for them to display in patient waiting areas.

**Table 2.1 GP Recruitment Prior to PCRN Adoption**

	Size of Practice <sup>a</sup>	COPD patients <sup>b</sup>	Letters sent <sup>c</sup>	Response	PIS sent out	Consented	Completed study
<b>Tokard Hill Medical Centre</b>	13 255	311	124	18 (15%)	13	9	3
<b>Stenhouse Medical Centre</b>	12 321	189	85	11 (13%)	7	5	2
<b>Highcroft Surgery</b>	11 636	214	84	12 (14%)	9	6	4
<b>Netherfield Medical Centre</b>	8 912	152	44	4 (9%)	4	0	0
<b>Barnby Gate, Newark</b>	13 238	183	88	5 (6%)	4	3	2
<b>West End Surgery</b>	6 491	166	29	2 (10%)	0	0	0
<b>Wilford Grove Surgery</b>	3 317	39	18	2 (11%)	2	1	0
<b>Church Walk Surgery</b>	11 261	295	133	14 (11%)	6	5	0
<b>Daybrook Health Centre</b>	9 012	187	*_	5	5	3	2
<b>Oakenhall Medical Practice</b>	7 067	159	83	12 (14%)	11	3	0
<b>Hucknall Road Medical Centre</b>	12 428	250	118	20 (17%)	15	9	2
<b>Zulu Road Medical Centre</b>	2 164	45	15	3 (20%)	3	1	1
<b>Ruddington Medical Centre</b>	*_	*_	*_	4	2	0	0
<b>Fairfields Practice</b>	6 165	123	52	4 (8%)	4	3	1
<b>Melbourne Park Medical Centre</b>	8 153	156	75	7 (9%)	7	2	1
<b>Leen View Surgery</b>	7 372	192	126	6 (5%)	5	2	1
<b>Orchard Medical Centre</b>	*_	*_	*_	5	4	3	2
<b>Rivergreen Medical Centre</b>	8 741	178	70	10 (14%)	5	1	0

**Key** - <sup>a, b</sup> – according to QoF database 2010 <sup>c</sup> - according to the Practice Manager \*- Not available **Abbreviation** – PIS: patient information sheet

**Table 2.2 GP Recruitment with PCRN Support**

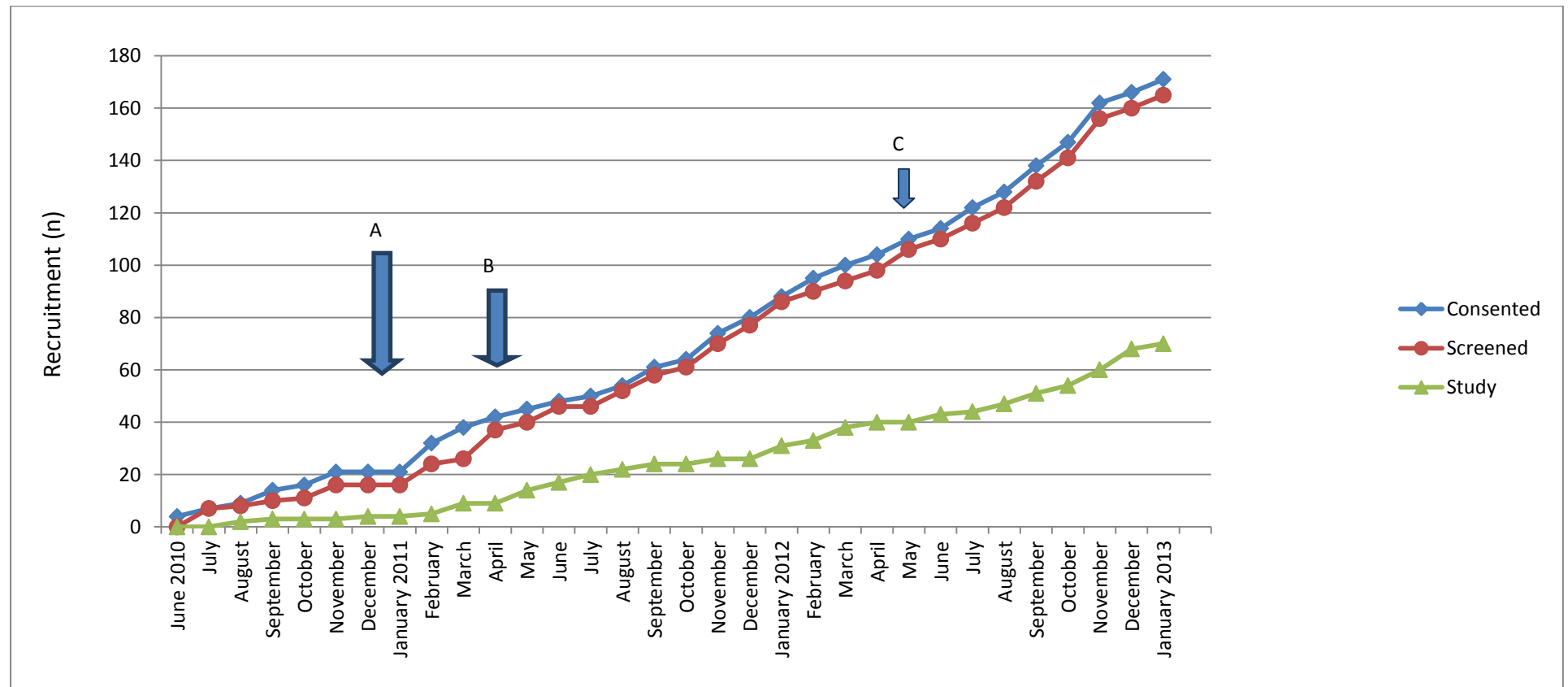
	<b>Letters sent<sup>c</sup></b>	<b>Patients replying</b>	<b>PIS sent out</b>	<b>Consented</b>	<b>Completed study</b>
<b>Blidworth Surgery</b>	37	5	5	4	1
<b>Moir Medical Centre</b>	14	1	1	1	0
<b>East Leake Health Centre</b>	31	7	4	2	1
<b>Churchside Medical Centre</b>	19	2	1	1	0
<b>Darley Dale Surgery</b>	21	2	2	2	0
<b>Limes Medical Centre</b>	53	4	3	2	1
<b>Wollaton Vale Health Centre</b>	5	2	2	1	1
<b>The Surgery at Wheatbridge</b>	98	14	11	7	3
<b>Lombard Medical Centre</b>	25	3	3	0	0
<b>Dronfield Medical Practice</b>	35	1	0	0	0
<b>Collingham Medical Centre</b>	29	4	4	3	3
<b>Rosemary Street Health Centre</b>	104	0	0	0	0
<b>Dr Sood and Partners</b>	40	2	2	0	0
<b>Newbold Surgery</b>	45	5	5	3	1
<b>Keyworth Medical Practice</b>	20	0	0	0	0
<b>Derby Road Health Centre</b>	31	6	5	4	0
<b>West Oak Surgery</b>	16	4	4	4	2
<b>Greenwood and Sneinton</b>	37	2	1	0	0

**Key - <sup>c</sup>** - according to the Practice Manager

**Abbreviation** – PIS: patient information sheet



**Figure 2.1 Cumulative Recruitment for the Statin RCT**



**Key** - A – Amendment applied for to change reversibility entry criteria, B – Approached GP practices to aid recruitment

C – PCRN started to help with recruitment

One of the main reasons for people failing screening was significant reversibility, with patients having a significant degree of reversibility to 400mcg of Salbutamol via inhaler and spacer. See Figure 3.2, p101. Initially the criteria was to exclude anybody who reversed by 12% or greater to 400mcg salbutamol. To coincide with guidelines an amendment was made and approved to add **>200ml** to the 12% as the cut-off for a significant bronchodilator response. The change in reversibility criteria did not affect the study design because it was not a primary outcome, and although reversibility results can help establish a diagnosis where uncertain, COPD guidelines are moving away from this. Moreover the guidelines recommend the use of post-bronchodilator spirometry in the diagnosis of COPD and acknowledge day-to-day variability in reversibility (Calverley et al., 2003).

#### **2.1.4      Patient Information Sheets**

Patient information sheets (PIS) were sent to everyone interested in the study that fulfilled the initial requirements, Appendix 1 page 222. When originally discussing the study with prospective patients' initial suitability could be assessed following simple questions such as checking diagnosis, medication and smoking history. Once a PIS had been sent out patients were phoned back after one week in order to allow time to receive the PIS and read it, to see if they were interested, and if they were, a consent visit was arranged at a mutually convenient time. All patient visits were in the Clinical Trials Unit (CTU) at City Hospital, Nottingham.

#### **2.1.5      Consent**

A consent visit was arranged to discuss the study with the patient and obtain informed consent. See appendix 2 page 234. This provided an opportunity to

answer any questions the patient had. If the patient agreed, a letter was sent to their GP to inform them of their patients' participation in the study.

#### **2.1.6      Screening**

The patients were then asked to attend a screening visit fasted for 8 hours and without having taken their inhaled medication – short acting bronchodilators withheld for 6 hours and long acting bronchodilators withheld for 12 hours.

They had their height and weight measured (methods 2.2.1 page 78), performed spirometry and reversibility (methods 2.2.3 page 82), had venepuncture to send off for measurements of their full blood count, fasting lipid profile and glucose levels, liver function and renal function (methods 2.2.7 page 87). Exhaled carbon monoxide levels were recorded (methods 2.2.12 page 91) and a capillary blood gas sample (methods 2.2.10 page 91) was taken for analysis of oxygen levels on air.

The most common reasons for subjects failing screening was significant reversibility, hypercholesterolaemia and unsuitable spirometry results.

#### **2.1.7      Drug Preparation**

The active medication (simvastatin) was purchased from Ranbaxy and shipped to Bilcare GCS (UK) who prepared the encapsulated statin and placebo. The simvastatin tablet was enclosed within a hard gelatine capsule, with lactose powder and magnesium stearate powder, which are excipients of the licensed product, used to prevent the tablet rattling within the capsule, and for the production of the placebo. The active treatment and placebo had identical appearance.

Disintegration tests on the over-encapsulated product were studied to show that the dosage form complied with pharmacopoeial requirements for disintegration. Although disintegration testing was performed, it could not be guaranteed that the statin works as it would do in its non-capsulated form.

#### **2.1.8      Dispensing**

Treatment was allocated by an independent pharmacist. 45 tablets were dispensed to each patient, whether active or placebo treatment. Patients were asked to take study medication in the evening, because this is when cholesterol biosynthesis is most active, hence greater reduction in cholesterol levels (Saito et al., 1991). The researcher did not have access to the randomisation codes. Pharmacy held them and the researcher would contact pharmacy in case of an emergency.

#### **2.1.9      Randomisation**

Each patient was randomly allocated to either active simvastatin treatment or a lactose based placebo treatment via a web based randomisation system. Randomisation was performed using a computer generated pseudo-random code, which used random permuted blocks of randomly varying size to produce a randomisation sequence. This was created by the Nottingham Clinical Trials Unit (NCTU) in accordance with their standard operating procedure (SOP) and held on a secure server. The sequence was generated using the stats package STATA and the function RALLOC as per local protocols. Randomisation was 1:1, stratified according to age group with categories 45-62 years and 63-80 years as age is an important variable of arterial stiffness (Laurent et al., 2006).

### **2.1.10      Study Visit 1**

Prior to visit 1 the subject was randomised, generating a prescription which was processed by the clinical trials pharmacy in preparation for the patient's visit. The patients were asked to attend visit 1 fasted for 8 hours and without having taken their short or long acting bronchodilator inhaled medication for 6 or 12 hours respectively. With-holding long-acting bronchodilators for 12 hours is not ideal, as some drugs have a longer duration of action than this. Tiotropium bromide (Spiriva), for example, has an onset of action of 30 minutes, with peak effect in 3-4 hours but a duration of action for  $\geq 24$  hours (Koumis and Samuel, 2005). Increasing the duration for subjects to with-hold long acting medication could have resulted in them taking short acting bronchodilators and therefore not meeting pre-visit requirements, or resulted in a bias towards those with milder disease.

Inclusion and exclusion criteria were re-checked. Height and weight were measured (methods 2.2.1), cardiovascular measurements were made including aortic stiffness (sphygmocor) and blood pressure (methods 2.2.2), bronchodilator administered; exhaled nitric oxide (FeNO) (methods 2.2.4), bioelectrical impedance (BIA) to determine body composition (methods 2.2.13), post-bronchodilator spirometry (methods 2.2.3), hand grip strength (methods 2.2.14) and carbon monoxide levels (methods 2.2.12) were performed, venepuncture (methods 2.2.7), six minute walking test (6MWT) (methods 2.2.9) and questionnaires completed (methods 2.2.15), spot urine sample collected, sputum induction (methods 2.2.5) and drug dispensed. Study visits always started with cardiovascular measurements and sputum induction was always performed last.

#### **2.1.11      During 6 Week Study Period**

Subjects were telephoned twice during the 6 week period, at 2 and 4 weeks post visit 1. This was to check compliance and for side effects. In addition, all subjects had access to a 24 hour a day number to phone and report any side effects or change in symptoms according to standard protocol for a Clinical Trial of an Investigational Medical Product RCT.

#### **2.1.12      Study Visit 2**

Study visit 2 was six weeks +/- 3 days post visit 1. The patients were asked to attend study visit 2 fasted and without having taken their inhaled medication as per study visit 1. Tests were performed as per study visit 1.

In addition the trial medication bottle was checked to count remaining tablets as a measure of compliance and related to time period from study visit 1. We defined good compliance as those subjects who took at least 90% of the medication for the days that they should have done, i.e. 6 weeks +/- 3days.

#### **2.1.13      Safety Profile**

A clinically relevant increase in Gamma-glutamyl transferase (gamma GT) and alanine transaminase (ALT) was defined as a value greater than 3 times the upper limit of normal. A clinically relevant increase in creatine phosphokinase (CPK) was defined as a value greater than 5 times the upper limit of normal.

Side effects classed as “musculoskeletal” included muscle aches and pains, cramps and joint aches. “Gastrointestinal” side effects included nausea, sickness and diarrhoea.

## **2.2 Procedures Used in Clinical Studies**

I was the sole member of staff appropriately trained in all procedures for the statin RCT and performed all tests according to local standard operating procedures (SOPs).

### **2.2.1 History and Examination**

For the statin RCT patients were questioned to make sure pre-visit instructions were followed, to ensure the patient had withheld inhaled therapy and was fasted. The inclusion and exclusion criteria were rechecked.

The subject's height in metres (m) and weight in kilograms (kg) were measured on a Seca (Hamburg, Germany) dual height and weight station bare-foot without shoes and outdoor clothing.

Smoking pack year history was calculated by dividing the number of cigarettes smoked per day by 20, and then multiplying that by the number of years smoked.

### **2.2.2 Haemodynamic Measurements**

Prior to commencing the statin RCT, there was a period of operator training. This involved spending 2 days in the Wales Heart Research Institute at the University of Wales Hospital, Cardiff, with health care professionals who regularly perform arterial stiffness measurements. After shadowing a few measurements and learning how to operate the machine I was observed performing measurements on willing subjects and deemed competent by my trainer. Following this 2 day training period I also performed measurements on willing participants before performing the tests on study patients.

The haemodynamic measurements include peripheral and central blood pressure recordings and measurements of pulse wave analysis (PWA) and PWV to assess arterial stiffness. Haemodynamic measurements were made in a quiet room at ambient temperature without bright lights.

#### *2.2.2.a Peripheral Blood Pressure*

Peripheral blood pressure was measured on the right arm using an Omron (705IT, UK) electronic sphygmomanometer in duplicate in the seated and supine position. Where the two readings exceeded 5mmHg difference a third measurement was made. The average of two repeatable measurements was recorded. For supine recordings, the subject was left to rest in the horizontal position for ten minutes prior to measurements.

Systolic and diastolic blood pressure readings were recorded in mmHg.

The peripheral mean arterial pressure (MAP) was a computed variable which was calculated using:

$$(1/3 \times \text{systolic blood pressure}) + (2/3 \times \text{diastolic blood pressure})$$

Central MAP was calculated on the machine software.

Peripheral and central pulse pressure (PP) was calculated using the equation:  
pulse pressure = systolic blood pressure – diastolic blood pressure.



### *2.2.2.b Pulse wave analysis and pulse wave velocity*

#### *2.2.2.b.i Sphygmocor*

Measurements for the statin RCT were performed using the sphygmocor® device.

The sphygmocor® (Atcor medical, Australia) measures central blood pressure, PWA and PWV using applanation tonometry to record waveforms (Laurent et al., 2006).

PWA provides measurements of central blood pressure, mean arterial pressure and augmentation index. Each measurement was recorded and the average of two repeatable results used.

Alx is an indirect measure of arterial stiffness and wave reflection (Laurent et al., 2006). To perform the PWA procedure using the Sphygmocor machine the radial pulse was first palpated, the probe was then placed over the area where the strongest pulse was felt. After a couple of seconds of repeatable good waveforms the data was captured and stored. See Appendix 5 page 246. The procedure is repeated following 10 minutes resting in the supine position. Measurements were always taken on the right hand side.

PWV is calculated using the time taken for the arterial waveform to travel through vasculature between 2 points of known distance apart. The gold standard for this measurement is carotid – femoral (aortic) PWV because it has better prognostic value (Laurent et al., 2006). For this study aortic PWV was performed as the primary variable and carotid – radial (brachial) was also recorded. At least two repeatable results were recorded and the average result for each measurement was used. To perform PWV a three lead electrocardiogram (ECG) was performed simultaneously with the procedure for PWA firstly at the carotid pulse, followed by

the femoral pulse for aortic PWV. The distance was measured in mm from the carotid pulse to suprasternal notch for the proximal distance, and the distance from the suprasternal notch to femoral pulse was measured in mm for the distal distance which were inputted into the machine prior to starting the test; see Appendix 6 p247 .

To perform brachial PWV the same procedure was done using the carotid pulse followed by the radial pulse. Again, two repeatable measurements were made and the average recorded. All PWV measurements were performed after resting in the supine position for at least 10 minutes.

One of the main causes of variation is path length measurements, and the method used to determine path length can significantly affect results (Sugawara et al., 2010). Differing proposed methods used to determine path length include the direct distance between the carotid and femoral pulses, the distance between the suprasternal notch (SSN) and femoral pulse with the SSN to carotid pulse distance subtracted, and the carotid to femoral pulse distance minus the SSN to carotid distance.

Measurements of arterial stiffness are operator dependent; however, to eliminate this all recording made for the RCT were performed by a single operator.

#### 2.2.2.b.ii Vicorder

Measurements for the 'Association of lung function and cardiovascular risk study' were made using the Vicorder (Skidmore Medical, UK), which is another non-invasive, oscillometric method of performing measurements of PWA, PWV and central blood pressure. Measurements of central blood pressure and PWA are

made in the seated and supine position. In a relaxed seated position a cuff was placed around the brachial pulse and inflated. When a steady trace was detected the results were saved and the procedure repeated until at least two measurements with an AIx within 5% were recorded. The average central blood pressure, MAP, AIx and HR were then taken. The procedure was repeated following 10 minutes rest in the supine position and the same measurements recorded. With this device brachial to femoral and carotid to femoral (aortic) measurements of arterial stiffness were made with simultaneous inflating cuffs over the respective pulses. The path length was measured as the distance between the midpoint on the brachial cuff to the midpoint on the femoral cuff in cm and the brachial PWV recorded, see Appendices 5-6. After 3 repeatable measures were made, the aortic PWV measurements were performed by simultaneously inflating a cuff around the carotid pulse with a cuff around the femoral pulse, using the distance measured between the mid-point of the carotid cuff and mid-point of the femoral cuff, see Appendices 5-6.

Repeatability of arterial stiffness measurements in patients with COPD using the vicorder device (Stone et al., 2013), Sphygmocor in healthy, hypertensive and hypercholesterolaemic (Wilkinson et al., 1998), and both vicorder and sphygmocor in patients with peripheral arterial disease (Shahin et al., 2013) has been reported.

### **2.2.3      Spirometry**

#### *2.2.3.a   Standard*

Spirometry was performed on a micro-medical spirometer (MicroLab MK6) in accordance with published ATS/ERS guidelines (Miller et al., 2005). The subject was

seated in an upright position with both feet flat on the floor. The chair did not have wheels but had arms for support. A maximal forced expiration was performed through the mouthpiece following maximal inspiration. Measurements of FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC ratio and peak expiratory flow (PEF) were recorded. Three repeatable results were recorded, defined as the FEV<sub>1</sub> and FVC being within 5% or 0.15l of each other (BTS/ARTP, 1994).

The obstructive ventilatory limitation caused by COPD is by definition, minimally or not reversible (Raherison and Girodet, 2009); and given the significant day to day variation in reversibility (Calverley et al., 2003), routine reversibility has been excluded from more recent guidelines (NICE, 2010a).

Reversibility was assessed during screening for the statin RCT and in the 'Association of lung function and cardiovascular risk' study, to identify those subjects with significant reversible airways and possible asthma. Reversibility testing should be reserved for cases where asthma and COPD cannot be differentiated – in these cases the National Institute for Clinical Excellence (NICE) guidelines recommend a 400ml improvement in FEV<sub>1</sub> from the baseline value (NICE, 2010a), whereas the American Thoracic Society/ European Respiratory Society (ATS / ERS) guidelines state a 12% and 200ml increase in FEV<sub>1</sub> and/or FVC from baseline shows a significant response (Pellegrino et al., 2005).

The arbitrary nature of the figures used to classify a significant response highlights the unreliability of bronchodilator responsiveness as an improvement to therapy (Calverley et al., 2003). For the statin RCT visits 1 and 2 spirometry was performed

post-bronchodilator (400mcg Salbutamol via metered dose inhaler and spacer) following the GOLD guidelines for COPD diagnosis and classification (GOLD, 2009).

Where spirometry was performed on more than one occasion, the same spirometer was used. Spirometers were verified using a 3 litre syringe prior to each testing session. Spirometry predicted values were calculated using the European Community of Coal and Steel (ECCS) equations (Quanjer et al., 1993).

#### *2.2.3.b Bronchodilator reversibility*

Reversibility was measured by performing baseline spirometry i.e. where the subject has not had any short acting inhaled bronchodilator medication for 6 hours or long acting bronchodilators for 12 hours, then administering 400mcg Salbutamol via a Metered Dose Inhaler (MDI) and spacer. Spirometry was repeated 20 minutes after administration, as this is when it will have maximum effect. Reversibility testing was only performed on subjects showing evidence of airflow obstruction on baseline spirometry.

#### *2.2.3.c Grading of airflow obstruction*

To report GOLD stage, the post-bronchodilator  $FEV_1\%$  predicted value were categorised: GOLD I (mild):  $FEV_1 \geq 80\%$ , GOLD II (moderate):  $50 \leq FEV_1\% < 80\%$ , GOLD III (severe):  $30 \leq FEV_1\% < 50\%$  and GOLD IV (very severe)  $FEV_1 < 30\%$  (Decramer et al., 2013); see Table 1.1. The statin RCT recruited only GOLD II-III, but the 'Associations of lung function and cardiovascular risk study' recruited all GOLD severities.

#### **2.2.4      Exhaled Nitric Oxide**

The fraction of exhaled nitric oxide (FeNO) measurement provides a quantitative measure of nitric oxide concentrations non-invasively, and is a useful biomarker to help distinguish between eosinophilic and non-eosinophilic inflammation (Pavord et al., 2008). Nitric oxide is produced in large and peripheral airways and alveoli by epithelial, endothelial and inflammatory cells (Barnes et al., 2010). NICE guidelines are in draft for the use of FeNO in asthma (NICE, 2013), but the potential role of FeNO in COPD has not been established yet.

FeNO was measured by steady exhalations at varying flow rates – 10, 30, 50, 100 and 200ml/s on a NIOX flex machine (Aerocrine, Sweden). Multi-flow measurements can be used to differentiate between alveolar and bronchial NO production and therefore distinguish the type of inflammation (Lehtimäki et al., 2001).

The test is performed by taking a maximal inspiration of NO (nitric oxide) free air through the mouthpiece to total lung capacity (TLC), then steadily exhaling at a constant rate also through the mouthpiece against a resistance. In those with significant airflow obstruction the test technique was not always possible to perform. Where possible, two measurements were recorded at each flow rate, and the mean used for analysis. The machine was calibrated every 14 days in accordance with manufacturer's guidance.

Alveolar gradient and bronchial intercept were not measured directly, but parameters estimated from a model such as the slope-intercept model (Tsoukias and George, 1998). The alveolar gradient corresponds to the alveolar

concentration and the bronchial intercept corresponds to the bronchial nitric oxide flux (Malinowski et al., 2009). The alveolar gradient and bronchial intercept refer to the regression line through the nitric oxide output for each of the flow rates.

A single flow rate technique can also be performed where measurements are just taken at the 50ml/s flow rate as this has been identified as a useful biomarker in patients with asthma (Barnes et al., 2010, Olin et al., 2007). We chose the five-flow rate method as little is known about FeNO and COPD it will provide the most information.

#### **2.2.5 Sputum Production and Induction**

Where possible, patients in the statin RCT provided spontaneous sputum samples. In those unable to produce an adequate spontaneous sample, sputum induction was performed.

Sputum was induced by inhaling increasing concentrations of saline solution – 3, 4 and 5% via a nebuliser (ultrasonic nebuliser NE-U17, Omron). Following each nebulisation FEV<sub>1</sub> was measured to check for any resulting bronchoconstriction. If FEV<sub>1</sub> dropped by 20% compared to pre-saline nebulisation the test would be stopped; if the drop was between 10-20% and the subject remained symptom free the test would continue but without increasing the saline concentration. As per departmental protocol, sputum induction was not performed on anyone with an FEV<sub>1</sub> <50% due to the risk of bronchoconstriction (Pizzichini et al., 2002).

#### **2.2.6 Sputum Processing**

Sputum plugs were isolated from saliva macroscopically. The resulting plugs were mixed with Dulbecco's Phosphate Buffered Saline (1:1) and protease inhibitor

(150µg per gram of isolated sputum) by vortexing (Fisher Scientific FB15012 TopMix) for 15 seconds before pulse sonicating for a further 15 seconds. The suspension was then filtered and a quota was removed to assess both cell number and viability by mixing with Trypan blue (1:1) and analysing using a haemocytometer. The remaining cell suspension was centrifuged (600g for 10 minutes at 4°C; Eppendorf 5702R) and the supernatant removed was frozen at -80°C to be used for the potential analysis of markers of airway inflammation. This was performed by GM and HB.

Differential sputum cell counts were determined manually by counting 400 non-squamous cells and was reported as relative numbers of eosinophils, neutrophils, macrophages, lymphocytes and epithelial cells; and then expressed as a percentage of total non-squamous cells. These were recorded at study visit one and study visit two, where possible.

### **2.2.7      Venous Blood Sample**

Venepuncture was performed using the aseptic non touch technique with the butterfly vacutainer system.

#### *2.2.7.a Pathology lab samples*

At each study visit of the statin RCT blood samples were taken to check safety measures, including LFT's and CPK.

Fasting lipids, liver function tests and CPK were measured on the Olympus AU2700 platform (Beckman Coulter, USA) according to manufacturer's settings.



### *2.2.7.b Centrifuging*

Serum separator blood bottles were centrifuged at least 30 minutes after the sample was taken at 1300g, 23°C for 10 minutes; and ethylenediaminetetraacetic acid (EDTA) blood bottles were centrifuged immediately after taking the blood sample at 1000g, 4°C for 15 minutes; aliquoted and stored at -80°C. All methods had been fully quality controlled prior to analysis in a CPA accredited laboratory.

### *2.2.7.c Research bloods – Statin RCT*

Blood samples were aliquoted and stored at -80°C for later analysis of inflammatory mediators.

#### *2.2.7.c.i Circulating hs-CRP*

High sensitivity CRP (hsCRP) was measured using an immuno-turbidimetric assay (Beckman Coulter) on an Olympus AU5400 analyser from a previously frozen (-80°C) serum sample and each sample was analysed once.

#### *2.2.7.c.ii Circulating MMP-9*

Total MMP-9 was measured using a quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, UK) in batches of stored aliquots of serum. The minimum detectable dose of MMP-9 is less than 0.156ng/ml, which was determined according to the product datasheet by adding 2SD to the mean optical density value of twenty zero standard replicates and calculating corresponding concentrations (RnDSystems, 2012). Each sample was measured in duplicate by WC.

#### *2.2.7.d Research bloods - Association of lung function and cardiovascular risk*

Blood samples were taken and stored for later analysis of serum AGE and serum soluble receptor for AGE (RAGE).

##### **2.2.7.d.i AGE**

Serum AGE was measured using an ELISA kit (Cell Biolabs, San Diego, USA) in batches of stored aliquots. Each sample was measured in duplicate by SS.

##### **2.2.7.d.ii RAGE**

Serum soluble RAGE was measured by WC using an ELISA kit (R&D systems, UK) in batches of stored aliquots of serum. Each sample was measured in duplicate.

#### **2.2.8 Skin Advanced Glycation End-products (AGE)**

Skin AGE was measured in the 'Association of lung function and cardiovascular risk' study using a non-invasive AGE Reader (DiagnOptics, Groningen, Netherlands). See Appendix 7 for a picture of the machine. This measurement was performed on Caucasian skin by placing the forearm (free from any creams or tanning lotion, bruising, scars or pigmentation) onto the AGE reader. Triplicate measures were made and then the average of the 3 recordings was taken as the final result.

A 4W UV-A emitting lamp is the light source from the AGE reader that illuminates 1cm<sup>2</sup> of the tissue of the forearm, with an excitation light source of between 300-420nm – peak excitation 350nm. Only light reflected from the skin is measured between 300-600nm with a spectrometer using a 200µm glass fibre. Dark and white reference readings were performed before each measurement to correct for background light and calculate reflectance; see Appendix 7. The degree of auto-

fluorescence (AF) was recorded (Meerwaldt et al., 2004, Mulder et al., 2006).

Autofluorescence was calculated as:

$$AF = \frac{\text{average light intensity emitted per nm over the 420-600nm range}}{\text{average light intensity emitted per nm over the 300-420nm range}}$$

### **2.2.9 Six Minute Walking Distance**

Six minute walking distance (6MWD) tests were performed to quantify the distance covered in order to assess exercise tolerance (Crapo et al., 2002). This test was performed post bronchodilator along a 10m straight walking course where the subject walks up and down a path of known distance, to assess functional ability. During the test subjects are not given any indication of the time i.e. how long they have walked for or how long they have left. Encouragement is limited and standardised to ensure consistency with each subject. Many previous studies report a practice test followed by a second test, as many subjects improve on the second attempt due to the learning effect (Sciurba et al., 2003). In this study we performed a practice walk, until the patient understood the requirements of the test, and was able to walk up the path and around the markers adequately. As the 6MWD were performed pre- and post-intervention in the statin study there was a six week gap between the two tests. This should have prevented any learning effects between the two results because of the time delay. The distance walked was recorded in metres and the pre- and post-exercise heart rate and oxygen saturations were also measured using pulse oximetry.

The current guidelines recommend a walking distance of 30m (Crapo et al., 2002) to perform the 6MWD test, however our subjects performed the test on a 10m path

due to restrictions within the department. The shorter walking distance may result in lower recorded 6MWD results, as the subjects have to turn around the course more often (Crapo et al., 2002). This was standardised however, as it was performed the same way for everyone.

#### **2.2.10      Capillary Blood Gases**

Capillary blood gases were performed to estimate the arterial oxygen ( $\text{PaO}_2$ ) and carbon dioxide ( $\text{PaCO}_2$ ) levels and pH. Subjects with hypoxaemia ( $\text{PaO}_2 < 7.3 \text{ kPa}$ ) were excluded. The earlobe was heated and vasodilated using Raljex® cream to increase blood perfusion. A blood sample was then collected in a capillary tube by scratching the earlobe with a lancet. Blood samples were analysed in the lung function department using a Radiometer ABL90 FLEX (Sussex, UK).

#### **2.2.11      Pulse Oximetry**

Pulse oximetry was performed using a Konica Minolta Pulsox-300 (Tokyo, Japan). This provides a measure of oxygen saturation and heart rate. The probe is placed on a finger nail bed free from any nail polish, with adequate circulation. A single measurement was made when there is adequate signal strength and the result is stable.

#### **2.2.12      Carbon Monoxide (CO) Measurements**

Exhaled carbon monoxide (CO) measurements were recorded as a quantifiable measure of CO as a combined result of smoking and atmospheric pollution. The measurements are made using a Clement Clarke Smokerlyzer CO monitor (Harlow, UK). To perform the test the subject inhales and breath-holds for 15 seconds,

before blowing out into the CO monitor. A reading was then displayed indicating the subjects CO levels.

#### **2.2.13      Body Composition**

The body mass index (BMI) was recorded from the Seca scales. Body composition measurements were recorded using a Tanita (Illinois, USA) bioelectrical impedance machine. Values of fat mass, percentage body fat, fat free mass percentage, total weight and resistance was recorded. These measurements were being performed due to the high reported prevalence of altered body composition in the COPD population. Fat mass index (FMI) was calculated as  $\text{fat mass}/\text{height}^2$  and the fat free mass index (FFMI) was calculated as  $\text{fat free mass}/\text{height}^2$ . Prior to performing the measurements the Tanita machine was set up with the patient's age, height and weight of clothes to allow for accurate readings. A standard weight of 0.5kg was used to account for clothes. To perform the test the participant stood with bare feet on the machine with a foot on either plate, after the initial weight measurement the subject will take hold of the handles, one in each hand for the body composition measurements.

BIA was contra-indicated in anyone with a pacemaker.

#### **2.2.14      Handgrip Strength**

Upper arm muscle strength was measured with handgrip strength using an analogue dynamometer (Takei, Japan). Three repeatable attempts are recorded with the dominant hand and encouragement was given during the test manoeuvre. The patients' dominant side was recorded. The average measurement for each hand was recorded.

## **2.2.15      Patient Questionnaires**

### *2.2.15.a MRC dyspnoea score*

The MRC score is the subjects' perception of their breathlessness, and is a score out of 5 (Fletcher, 1960), see Appendix 8. The scale ranges from 1 – 'not troubled by breathlessness except on strenuous exercise' to 5 – 'too breathless to leave the house, or breathless when dressing or undressing'. The higher the score the more severe the subject perceives their breathlessness.

### *2.2.15.b COPD Assessment Test (CAT) score*

The CAT score is a self-reported questionnaire with 8 evenly weighted questions used to assess the impact of COPD upon the subject (Jones et al., 2009). Each question is scored between 0 (no impact) and 5 (very severe impact), giving CAT total scores of between 0 and 40. The higher the subject scores, the more impact COPD is having on their daily lives; see Appendix 9.

### *2.2.15.c St George's Respiratory Questionnaire (SGRQ)*

The impact of disease upon the patient's quality of life is assessed using the self-reported St Georges Respiratory Questionnaire (Jones et al., 1992), see Appendix 10. SGRQ results were analysed and a score for the domains of symptoms, activity, impact and a total score was recorded.

## **2.2.16      Funding**

The research presented in this thesis was initially supported by the University of Nottingham and the Nottingham Respiratory BRU. The statin RCT was funded through a National Institute for Health Research (NIHR) Research for Patient Benefit grant (RfPB). I was subsequently awarded an NIHR fellowship BRF-2011-012.

# **Chapter 3**

## **The Cardiovascular Effects of Simvastatin in Patients with COPD:**

**A double blind, randomised,  
placebo controlled trial**

### **3 The Cardiovascular Effects of Simvastatin in**

### **Patients with COPD: A double blind, randomised,**

### **placebo controlled trial**

#### **3.1 Introduction**

With the recent interest in cardiovascular (CV) disease in subjects with COPD, and the prevention and treatment of such comorbidities (Decramer et al., 2013, Fabbri et al., 2008), it is surprising that other than smoking cessation, routine COPD management is not focused on preventing CV disease.

Aortic stiffness is an independent predictor of CV risk (Laurent et al., 2001), and is increased in COPD subjects, over and above the effects of smoking (Sabit et al., 2007, Maclay et al., 2009). Aortic PWV has been reported to predict both future fatal and non-fatal CV events, and mortality; even after adjusting for established CV risk factors (Ben-Shlomo et al., 2013).

Aortic PWV is responsive to intervention over a short period of time. Statins ability to modulate arterial stiffness has been shown in people with other chronic illnesses (Meng et al., 2009, Maki-Petaja et al., 2007) as described in chapter 1.

Statins are not currently recommended for primary prevention of CV disease in patients with COPD, despite statins being shown in other chronic diseases with an increased CV risk to have a role in CV prevention (Colhoun et al., 2004). In subjects with normal LDL-cholesterol levels, but increased CRP levels, rosuvastatin 20mg daily significantly reduced CV events in apparently healthy subjects (Ridker et al.,



2008). People with diabetes also have an increased risk of CV disease, and a study reported that the use of atorvastatin 10mg daily in the diabetic population with normal LDL-cholesterol levels and no history of CV disease was effective in reducing first CV events, and did so by 37% (Colhoun et al., 2004).

Statins have been shown through population based retrospective cohort studies to be cardio-protective in COPD patients irrespective of individual CV risk and steroid use (Mancini et al., 2006). Other smaller studies have been done which support these findings (Soyseth et al., 2007, Lee et al., 2008), but there are no RCT's with CV outcomes. A prospective observational study found that long term statin use, defined as at least 2 years, decreases all-cause mortality in COPD by 39%; whilst short term statin use, defined as at least 30 days, decreases respiratory mortality by 64% by modulating the baseline level of systemic inflammation (Lahousse et al., 2013). In a matched cohort study, moderate dose statin users – defined as  $\geq$  4mg/day, were found to have a significantly lower COPD mortality and a reduced risk of death from influenza/pneumonia (Frost et al., 2007).

To date, there are no RCT's of statins in COPD with CV outcome measures. Indeed, until recently there have been no RCTs of the effects of medication in COPD on primary cardiovascular endpoints, with only observational or retrospective analyses of RCT's which had primary lung endpoints. A recent study by Dransfield *et al.* provides the first RCT evidence of inhaled medication in COPD and CV outcomes (Dransfield et al., 2011).

**Hypothesis:**

Increased aortic stiffness, as measured by aortic PWV, would be lowered following 6 weeks treatment of simvastatin 20mg once daily compared to placebo in patients with COPD, but without concurrent cardiovascular disease, hypercholesterolemia or diabetes.

## 3.2 Methods

This study was a double-blind, placebo controlled RCT, performed at a single centre - the Nottingham Respiratory Research Unit, City Hospital, Nottingham. All ethical, R&D and MHRA approvals were sought prior to commencement – see methods 2.1.1.

### 3.2.1 Recruitment

In order to fulfil enrolment requirements 633 subjects expressed an interest from our recruitment efforts, with 504 of these appearing suitable following telephone conversation and interested in receiving the PIS; see Table 3.1 and Figure 3.1.

Subjects with COPD were recruited into the study as described in methods 2.1.3 p68, following detailed inclusion and exclusion criteria:

*Inclusion criteria:* as previously described – see methods 2.1.2 p66, but most pertinently a current or ex-smoker with an appropriate smoking history, aged between 45 and 80 years of age with confirmed COPD.

*Exclusion criteria:* The full exclusion criteria are in the methods chapter, see chapter 2 p66. The key exclusion criteria pertinent to the population were not currently taking a statin, no hypercholesterolemia, no CV disease and not diabetic.

The reasons for failing screening are presented in Figure 3.2.

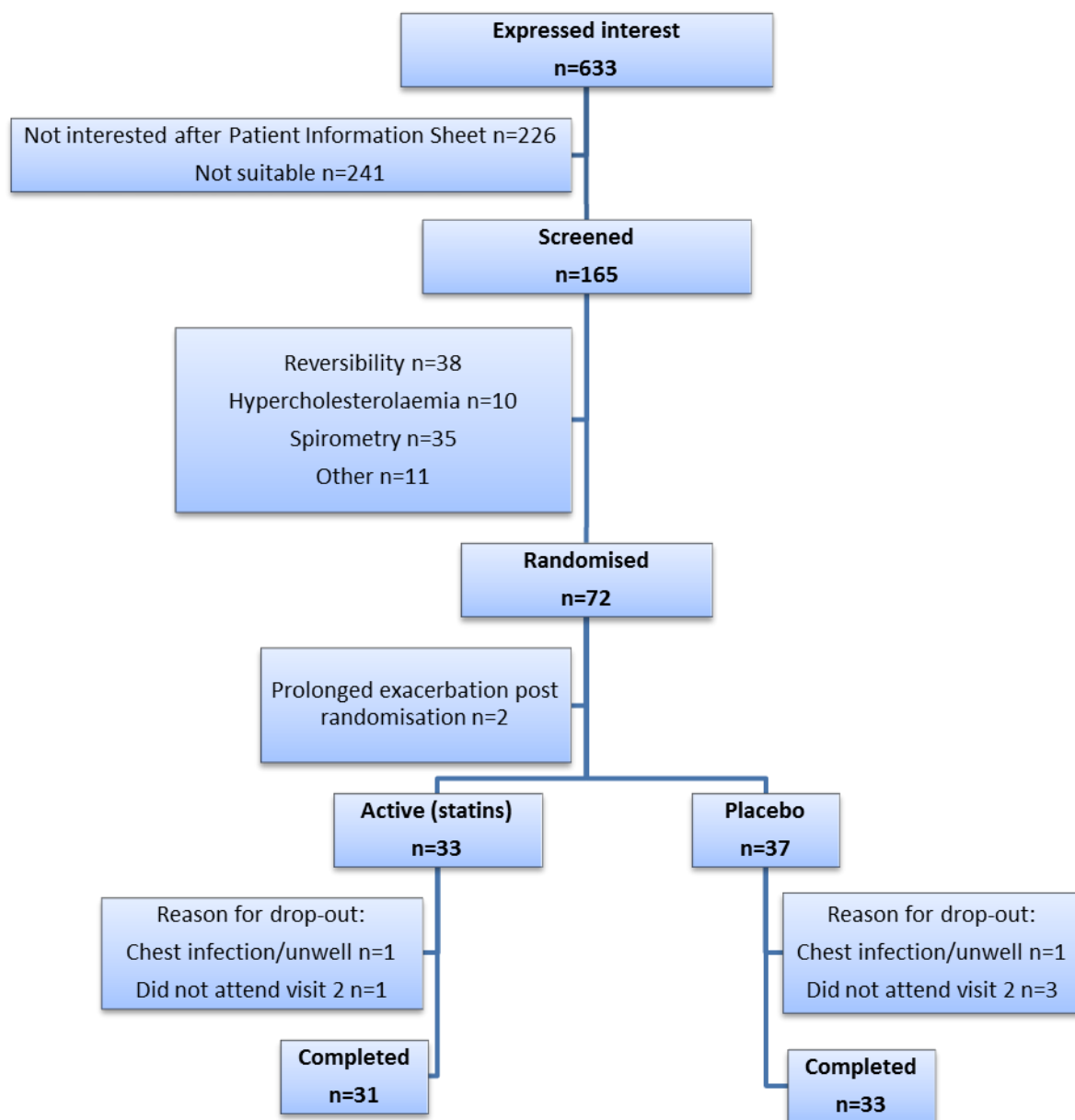
**Table 3.1 Individual and Cumulative Recruitment for the Statin RCT**

**Table a: Recruitment figures**

**Table b: Cumulative Recruitment figures**

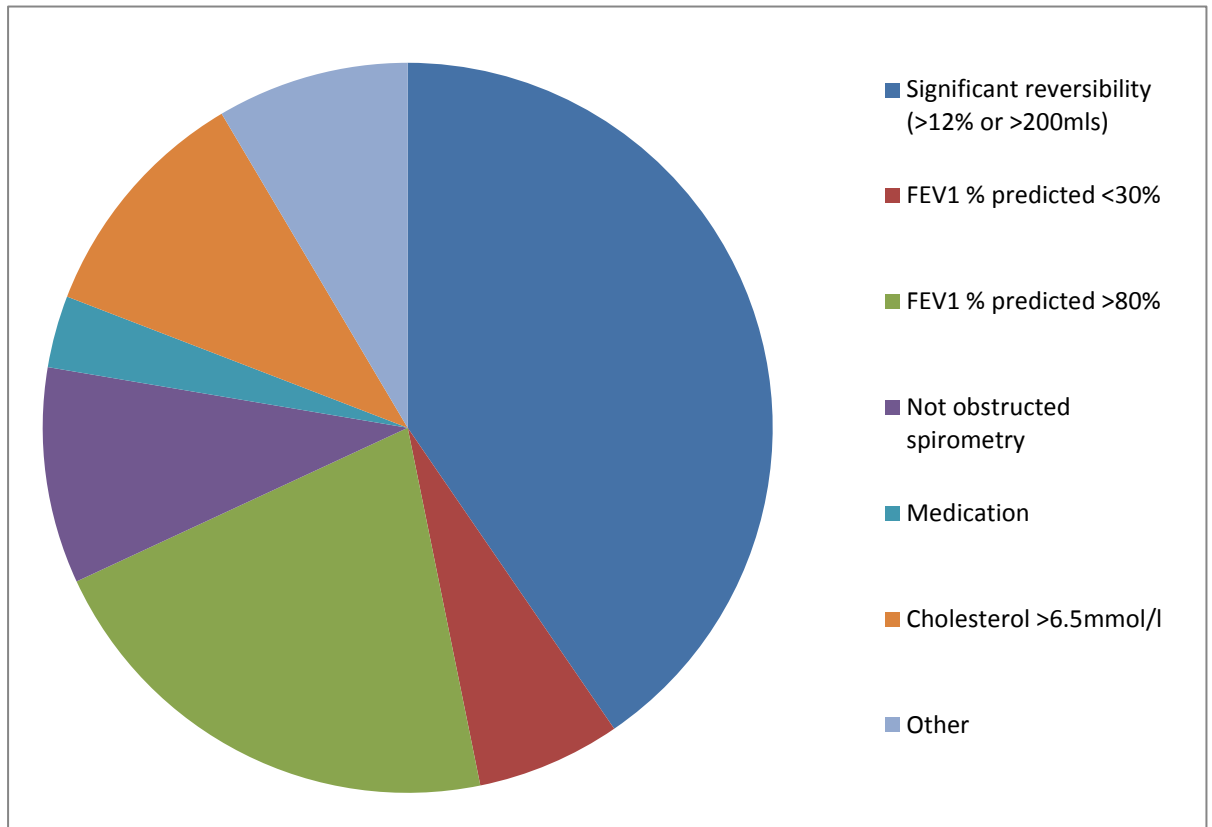
	Consented	Screened	Study			Consented	Screened	Study
June 2010	4	0	0		June 2010	4	0	0
July	3	7	0		July	7	7	0
August	2	2	2		August	9	9	2
September	5	2	1		September	14	11	3
October	2	4	0		October	16	15	3
November	5	5	0		November	21	20	3
December	0	0	1		December	21	20	4
January 2011	0	0	0		January 2011	21	20	4
February	11	10	1		February	32	30	5
March	4	4	4		March	36	34	9
April	7	3	0		April	43	37	9
May	3	5	5		May	45	40	14
June	3	6	3		June	48	46	17
July	2	0	3		July	50	46	20
August	4	6	2		August	54	52	22
September	7	7	1		September	61	59	23
October	3	3	1		October	64	62	24
November	10	11	2		November	74	73	26
December	6	7	0		December	80	77	26
January 2012	8	9	5		January 2012	88	86	31
February	7	4	2		February	94	90	33
March	5	4	5		March	100	94	38
April	4	4	2		April	104	98	40
May	6	8	0		May	110	106	40
June	4	4	3		June	114	110	43
July	8	6	1		July	122	116	44
August	6	6	3		August	128	122	47
September	10	10	4		September	138	132	51
October	9	9	3		October	147	141	54
November	15	15	6		November	162	156	60
December	4	4	8		December	166	160	68
January 2013	5	5	2		January 2013	171	165	70

**Figure 3.1 Consort Diagram of Study Recruitment, Allocation, Drop-out and Completion**



‘Other’ reasons for failing the screening visit included cardiac history, alcohol excess, maintenance prednisolone/antibiotics, withdrew consent, other respiratory diagnosis, never smoker and outside age range.

**Figure 3.2 Reasons for Failure at Screening Visit (n=failures)**



### **3.2.2      Consent Visit**

Interested patients, after reading the PIS, were invited for a consent visit to obtain informed written consent, see methods 2.1.5 p73.

### **3.2.3      Screening**

During the screening visit eligibility was checked. A full history was taken. Spirometry, including bronchodilator reversibility was performed, see methods 2.2.3 p74. Fasting lipid profile, liver function test (LFT), renal function, glucose and full blood count were measured, see methods 2.2.7 p87. Carbon monoxide was measured, see methods 2.2.12 p91.

### **3.2.4      Randomisation**

Eligible subjects that were recruited were then randomised, see methods 2.1.9 p75.

### **3.2.5      Study Visit 1**

During study visit one the baseline data was collected. The primary variable was aortic PWV, which was measured to assess aortic stiffness using the sphygmocor, see 2.2.2.b p80. Other tests performed pertinent to this chapter were post-bronchodilator spirometry, haemodynamic measurements, venepuncture – safety bloods (LFT and creatine phosphokinase (CPK)), lipid profile and high sensitivity C-reactive protein (hs-CRP); CO and 6MWD. See methods 2.1.10 p76.

### **3.2.6      Treatment Phase**

Telephone calls were made to each patient, see methods 2.1.10

### **3.2.7      Study Visit 2**

Post-treatment results were recorded as per study visit one. See methods 2.1.10 p76. Compliance was checked and recorded.

### **3.2.8      Data Analysis**

Data was analysed using Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 21.0. A  $p < 0.05$  was considered significant. The normality of the data was checked graphically. Graphical interpretation was made using frequency distribution histograms and Q-Q plots. Unless otherwise specified, arithmetic mean and standard deviation was used to show the measure of central tendency.

Analysis: The primary outcome measure was aortic stiffness, using the parameter aortic PWV and was analysed using a general linear regression model, incorporating treatment group. The aortic PWV was measured in m/s and can be influenced by BP and age, and to a lesser extent sex. Post-treatment measurements were made at 6 weeks +/- 3days whilst the patients were still on active/placebo.

The primary analysis was to compare the difference in the mean difference of the active and placebo arms for aortic PWV using an unpaired t-test.

A secondary stratified analysis was then performed including baseline characteristics that were thought to influence the outcome; such as baseline PWV, age, sex, change in mean arterial pressure (MAP) and baseline total cholesterol levels. This did not include the age stratification as age is not thought to influence the effect of statins on the change in PWV - only the baseline aortic PWV.



Secondary variables analysed were seated and supine/peripheral and central systolic, diastolic, mean arterial and pulse pressure; plus augmentation index, heart rate and carotid-radial PWV.

A pre-defined subgroup analysis was performed to compare the mean change in difference of the active and placebo arms for aortic PWV in the subgroup of patients with a baseline aortic PWV measurement of >10m/s. A value of 10m/s was determined prior to data analysis because it was deemed to be the lowest acceptable value to use as a cut-off for increased arterial stiffness, and has been proposed as the cut-off value for aortic PWV (Van Bortel et al., 2012).

The 10m/s cut-off point is based on previous guidance to use 12m/s (Mancia et al., 2007), which has been adapted to account for the new path length measurement recommended for use during the aortic PWV procedure which is now of 80% of the distance from the common carotid artery to the common femoral artery as opposed to 100% as previously used, and has been adopted in the latest ESH-ESC guidelines (Mancia et al., 2013).

### 3.3 Results

Of the 633 subjects that expressed an interest in the study 241 were found to be not suitable, for example if they mentioned a pre-existing condition or lack of smoking history during initial telephone conversation. A further 221 were no longer interested, or were unable to be re-contacted after receiving the PIS. This left 171 subjects to be consented of whom 165 were screened, see Figure 3.1. From this, 72 people were suitable and randomised. One subject was randomised twice as they had repeated acute exacerbation of COPD (AECOPD) post-randomisation; so whilst fulfilling the criteria, was then unsuitable to start the study and subsequently withdrew consent. Therefore, although 72 subjects were randomised, only 70 were prescribed study medication and started the RCT.

From the 70 subjects that started the study, 33 were randomised into the active arm of the study and 37 into the placebo arm. Of these, 31 subjects in the active group completed the study and 33 from the placebo group.

#### 3.3.1 Demographics

64 subjects completed the study out of the 70 randomised and starting the study medication. No follow on data is available for those who dropped out of the study.

Baseline data for those on active treatment are presented in Table 3.2, alongside the placebo group. There was no evidence of significant hypoxemia in either group.

At baseline the number of subjects in each of the aortic PWV groups (<8m/s, 8-10m/s and >10m/s) was: <8m/s n=21 (30%), 8-10m/s n=24 (34%) and >10m/s n=25 (36%).

**Table 3.2 Baseline Demographics for the Statin RCT**

<b>Mean (SD) Unless otherwise specified (t-test unless otherwise specified)</b>	<b>Active group n=33</b>	<b>Placebo group n=37</b>
<b>Age (years) Median (range)</b>	64 (50-79)	65 (50-79)
<b>Sex Male n (%) *</b>	26 (79)	24 (65)
<b>Smoking status (Current:Ex) n(%) *</b>	7(21):26(79)	15(41):22(59)
<b>Smoking pack years</b>	46 (25)	48 (22)
<b>FEV<sub>1</sub> (L)</b>	1.6 (0.6)	1.4 (0.5)
<b>FEV<sub>1</sub> %predicted</b>	55 (14)	53 (15)
<b>FEV<sub>1</sub>/FVC</b>	46 (12)	47 (12)
<b>GOLD stage n(II, III)</b>	20, 13	22, 15
<b>Exhaled CO (ppm)</b>	3 (3)	5 (4)
<b>Total cholesterol (mmol/L)</b>	5.3 (0.8)	5.4 (0.8)
<b>HDL cholesterol (mmol/L) †</b>	1.6 (1.3)	1.7 (1.3)
<b>LDL cholesterol (mmol/L)†</b>	2.9 (1.3)	2.9 (1.3)
<b>SGRQ total</b>	38 (17)	38 (19)
<b>MRC dyspnoea score Median (range)</b>	2 (1-5)	2 (1-5)
<b>Resting SpO<sub>2</sub> on air (%) Median (range)</b>	95 (92-98)	95 (90-98)

† geometric mean \*chi-squared test

**Abbreviations** – **FEV<sub>1</sub>**: forced expired volume in 1 second; **FVC**: forced vital capacity; **GOLD**: Global initiative for chronic Obstructive Lung Disease; **CO**: carbon monoxide; **HDL**: high density lipoprotein; **LDL**: low density lipoprotein; **SGRQ**: St George's Respiratory Questionnaire; **MRC**: Medical Research Council; **SpO<sub>2</sub>**: oxygen saturation levels as measure by pulse oximetry

### **3.3.2      Drop Outs**

There were no apparent differences in baseline lung function – FEV<sub>1</sub> and FEV<sub>1</sub>% predicted and aortic PWV between those who dropped out (n=6) and those who completed the study (n=64). For the subjects that dropped out of the study baseline mean (standard deviation (SD)) was- FEV<sub>1</sub>: 1.64 (0.66) L, FEV<sub>1</sub>% predicted 56 (13) and aortic PWV 10.1 (3.6) m/s.

### **3.3.3      Aortic PWV**

Of the 64 subjects that completed the study, aortic PWV measurements were only measured in n=63, but n=70 at baseline. Central blood pressure measurements were in n=62, because accurate results could not be obtained in everyone due to technical challenges.

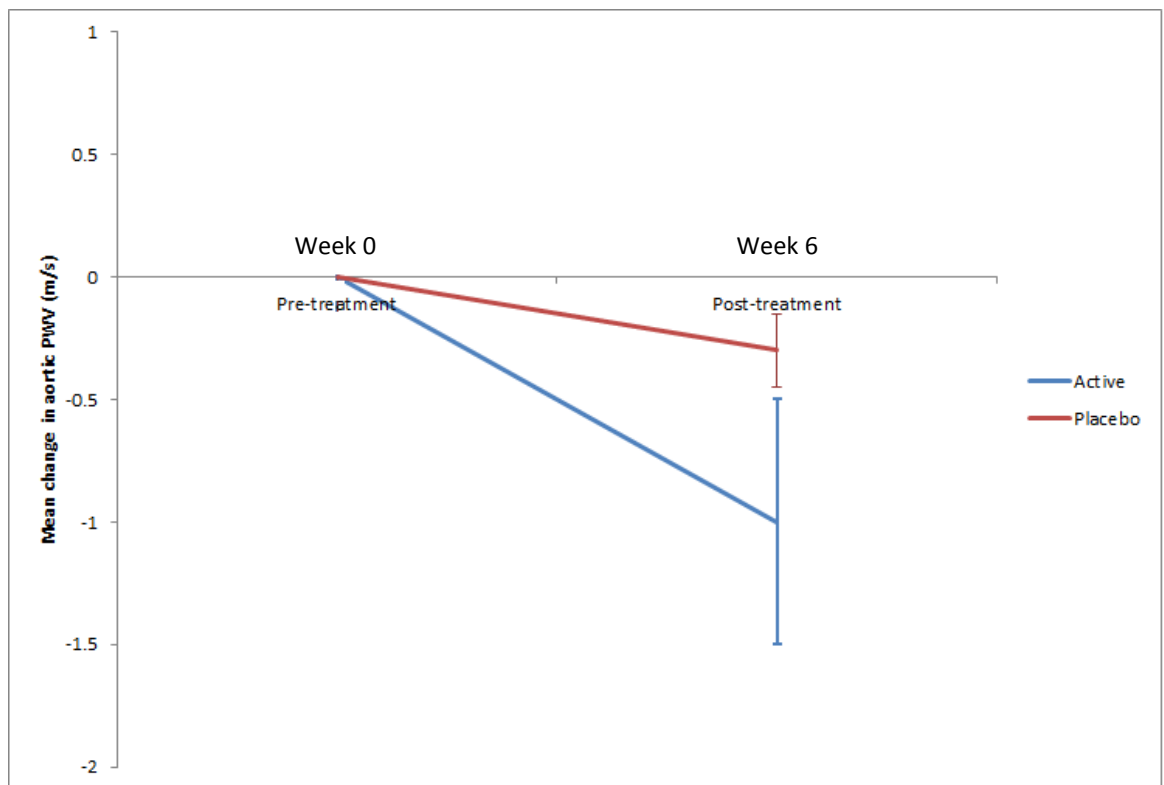
There was a drop in aortic PWV of -1.0m/s in the active arm, p=0.06 over 6 weeks; see Figure 3.3. Therefore, we did report the required change of what the study was powered to, but the placebo group also had a drop in aortic PWV following treatment and although there was a larger drop in aortic PWV in the active arm compared to placebo, the difference in change between arms was not significant: -0.7 (-1.8, 0.5)m/s, see Table 3.3. Adjusting for confounding factors in a secondary stratified analysis with the primary variable of the linear regression being change in aortic PWV, and the confounding variables which were adjusted for were age, sex, change in MAP and then cholesterol levels; using an entered regression model did not alter this. Table 3.3 demonstrates the primary and secondary outcome measures pre- and post- treatment for both the active (simvastatin) and placebo group.

**Table 3.3 Cardiovascular Measures for all Subjects Pre- and Post-treatment**

Mean (SD) n=total (active: placebo)		Active (simvastatin) group			Placebo group			Mean difference Δ active versus Δ placebo (CI)
		Pre – treatment	Post – treatment	Post-Pre Mean 'Δ'	Pre – treatment	Post – treatment	Post-Pre Mean 'Δ'	
Aortic PWV (m/s) n=63 (31:32)		9.9 (3.4)	8.9 (2.2)	-1.0	9.1 (2.3)	8.9 (3.0)	-0.3	-0.7 (-1.8, 0.5)
Brachial PWV (m/s) n=31 (12:19)		8.9 (1.8)	8.3 (1.4)	-0.4	7.7 (1.4)	7.5 (1.6)	-0.2	-0.2 (-1.5, 1.2)
Total cholesterol levels(mmol/l) n=64 (31:33)		5.2 (0.8)	4.1 (0.8)	-1.2	5.4 (0.8)	5.3 (0.9)	-0.1	-1.1 (-1.3, -0.8)
Triglycerides (mmol/l) n=64 (31:33)		1.2 (0.6)	1.1 (0.5)	-0.2	1.5 (1.4)	1.5 (1.2)	-0.1	-0.1 (-0.4, 0.2)
HDL cholesterol (mmol/l) n=62 (31:31)		1.6 (1.3)	1.7 (0.4)	0.0	1.7 (1.3)	1.7 (0.5)	0.0	0.1 (-0.1, 0.2)
LDL cholesterol (mmol/l) n=62 (31:31)		2.9 (0.7)	1.9 (0.6)	-1.1	3.0 (0.8)	2.9 (0.8)	0.0	-1.0 (-1.3, -0.8)
Peripheral Seated n=64(31:33)	Systolic BP (mmHg)	147 (22)	144 (18)	-3.1	151 (25)	145 (24)	-5.5	2.4 (-3.7, 8.6)
	Diastolic BP (mmHg)	90 (12)	87 (11)	-3.6	88 (14)	86 (12)	-2.4	-1.3 (-4.7, 2.1)
	MAP (mmHg)	109 (14)	106 (12)	-3.5	109 (16)	105 (15)	-3.4	-0.1 (-3.8, 3.6)
	Pulse pressure (mmHg)	57 (15)	58 (16)	0.6	63 (18)	60 (19)	-3.1	3.7 (-1.4, 8.8)
Central Seated n=62(30:32)	Systolic BP(mmHg)	137 (18)	135 (22)	-1.6	138 (23)	133 (23)	-4.9	3.3 (-2.5, 9.0)
	Diastolic BP (mmHg)	91 (12)	86 (12)	-4.1	89 (14)	85 (14)	-2.2	-1.9 (-5.5, 1.7)
	MAP (mmHg)	111 (14)	108 (12)	-3.3	110 (18)	107 (16)	-3.3	-0.1 (-4.1, 4.0)
	Pulse pressure (mmHg)	46 (12)	49 (16)	2.5	49 (16)	47 (19)	-2.7	5.2 (0.2,10.1)
	Heart rate (bpm)	73 (13)	73 (14)	-0.4	74 (15)	76 (17)	2.3	-2.7 (-6.1, 0.7)
	Augmentation Index (%)	27.4 (8.7)	26.6 (8.9)	-0.9	27.5 (10.4)	25.9(10.3)	-2.3	1.4 (-2.0, 4.9)
Peripheral Supine n=64(31:33)	Systolic BP(mmHg)	144 (24)	141 (22)	-3.4	144 (24)	142 (23)	-1.4	-2.0 (-8.5, 4.4)
	Diastolic BP(mmHg)	86 (12)	84 (12)	-1.5	82 (13)	83 (13)	1.7	-3.2 (-6.9, 0.5)
	MAP (mmHg)	105 (15)	103 (14)	-2.1	102 (15)	103 (15)	0.7	-2.8 (-7.1, 1.4)
	Pulse pressure (mmHg)	58 (16)	56 (16)	-1.9	62 (18)	59 (18)	-3.1	1.2 (-3.5, 5.9)
Central Supine n=58(27:31)	Systolic BP (mmHg)	136 (21)	135 (22)	-0.8	134 (22)	133 (23)	-2.0	1.3 (-5.7, 8.2)
	Diastolic BP (mmHg)	87 (13)	86 (12)	-1.3	84 (13)	85 (14)	0.8	-2.1 (-6.0, 1.8)
	MAP (mmHg)	108 (16)	107 (15)	-0.9	105 (16)	104 (18)	-1.1	0.2 (-5.0, 5.4)
	Pulse pressure (mmHg)	49 (13)	49 (16)	0.5	50 (16)	48 (16)	-2.9	3.4 (-0.8, 7.6)
	Heart rate (bpm)	68 (11)	68 (12)	-0.7	71 (13)	73 (15)	2.2	-2.9 (-6.5, 0.7)
	Augmentation Index (%)	29.7 (8.0)	28.7 (7.5)	-1.1	30.2 (9.8)	28.1(10.7)	-2.5	1.3 (-2.7, 5.4)

**Abbreviations** - PWV: pulse wave velocity, HDL: high density lipids, LDL: low density lipids, BP: blood pressure, MAP: mean arterial pressure

**Figure 3.3 Change in Aortic PWV After Treatment with Simvastatin or Placebo**



Mean change in aortic PWV (m/s) from baseline following 6 weeks treatment with active (simvastatin) or placebo.

Error bars = standard error.

### **3.3.4      Other Haemodynamic Measures**

There was no difference in change between arms in brachial PWV (n=33), p=0.81 or AIx (n=62) in either seated or supine measurements: p=0.40 and p=0.52 respectively. There were no significant changes in central or peripheral systolic, diastolic or mean arterial blood pressure, or pulse pressure measurements. See Table 3.3.

### **3.3.5      Cholesterol Levels**

Total cholesterol and LDL cholesterol levels decreased in the active group compared to placebo group post treatment, p<0.001 respectively. See Table 3.3.

There was no correlation between baseline total cholesterol or LDL-cholesterol level and baseline aortic PWV.

### **3.3.6      Compliance**

When accounting for the visit 2 date i.e. 6 weeks from visit one +/- 3 days, the active group did not take a mean (SD) of 0.7 (1.0) tablets and the placebo group 1.4 (2.0) tablets, p=0.06. When good compliance was defined by taking at least 90% of the study medication, there were 15 subjects with poor compliance: placebo n=10, active n=5. All patients took a minimum of 36 tablets.

### **3.3.7      Subgroup Analysis: Aortic PWV >10m/s**

A sub-group analysis of subjects with baseline aortic PWV >10m/s was performed. This included n=25: active n=12 and placebo n=13. The active and placebo group were well matched for age, sex, smoking history and baseline haemodynamic, lung function and cholesterol results; see Table 3.4.

Mean (SD) aortic PWV for the total subgroup was 12.6 (2.5) m/s. In keeping with the main group there were a higher proportion of males and ex-smokers in both the active and placebo sub-groups. The sub group was marginally older than the main group. Smoking history, lung function and cholesterol levels were comparable with the main group.

There was a significant difference in the change between the active arm of this subgroup  $n=11$  and the placebo arm  $n=11$ ,  $p=0.03$  for aortic PWV; see Figure 3.4 and Figure 3.5 and Table 3.5. This significant difference remained after adjusting for age, sex and change in MAP in a stepwise linear regression,  $p=0.009$ .



**Table 3.4 Sub-group Analysis in those with a Baseline Aortic PWV >10m/s: Baseline Demographics**

<b>Mean (SD) Unless otherwise specified (t-test unless otherwise specified)</b>	<b>Active (simvastatin) n=12</b>	<b>Placebo n=13</b>
<b>Age (years) Median (range)</b>	70 (54-77)	64 (52-75)
<b>Sex Male n (%) *</b>	9 (75)	10 (77)
<b>Smoking status (Current:Ex) n(%) *</b>	4(33):8(67)	5(38):8(62)
<b>Pack years</b>	46 (23)	46 (23)
<b>Aortic PWV (m/s)</b>	13.2 (2.9)	12.0 (2.1)
<b>Peripheral systolic BP (mmHg)</b>	156 (27)	159 (31)
<b>Peripheral diastolic BP (mmHg)</b>	94 (14)	90 (18)
<b>FEV<sub>1</sub> (l)</b>	1.4 (0.6)	1.6 (0.7)
<b>FEV<sub>1</sub> %predicted</b>	51 (13)	55 (17)
<b>FEV<sub>1</sub>/FVC</b>	41 (8)	48 (10)
<b>Total cholesterol (mmol/L)</b>	5.3 (0.8)	5.3 (0.8)
<b>HDL cholesterol (mmol/L)</b>	1.7 (0.4)	1.7 (0.5)
<b>LDL cholesterol (mmol/L)</b>	3.0 (0.6)	2.8 (0.7)
<b>SGRQ total</b>	43 (16)	35 (20)
<b>Resting SpO<sub>2</sub> on air (%) Median (range)</b>	95 (93-98)	95 (90-97)

† geometric mean \* chi-squared test

**Abbreviations** – **BP**: blood pressure; **FEV<sub>1</sub>**: forced expired volume in 1 second; **FVC**: forced vital capacity; **HDL**: high density lipoprotein; **LDL**: low density lipoprotein; **PWV**: pulse wave velocity; **SGRQ**: St George's Respiratory Questionnaire; **SpO<sub>2</sub>**: Oxygen saturation levels as measure by pulse oximetry

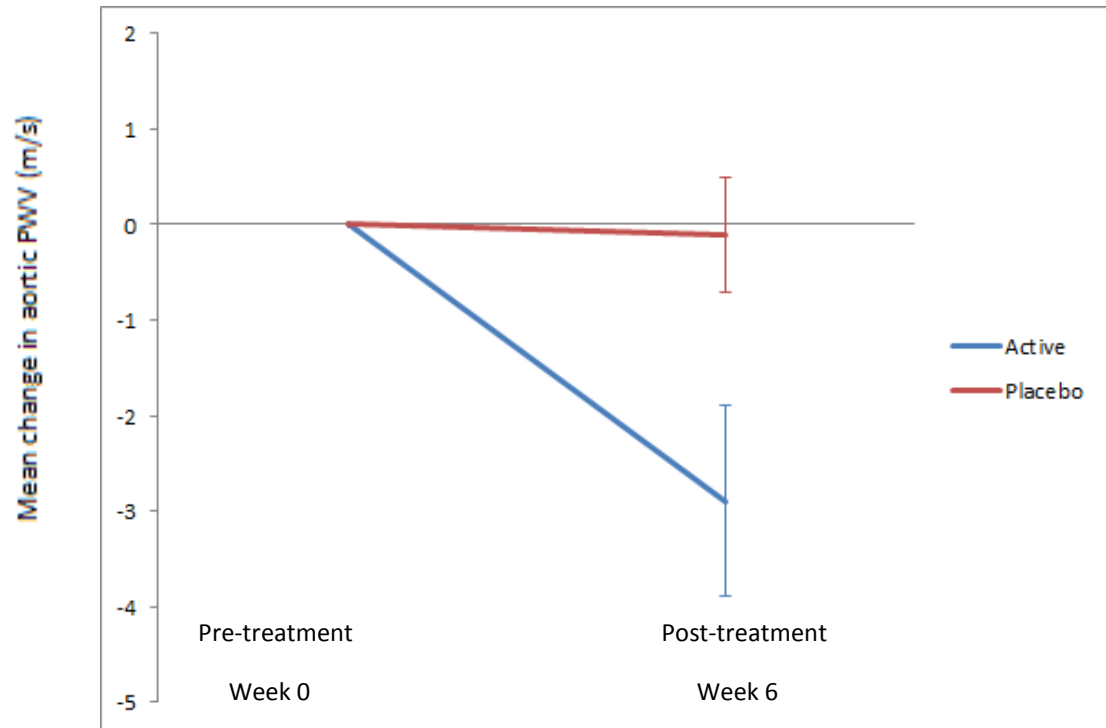
**Table 3.5 Subgroup Analysis in those with a Baseline Aortic PWV >10m/s: Cardiovascular Markers**

Mean (SD) n=total (active: placebo)	Active		Placebo		Mean difference $\Delta$ active versus $\Delta$ placebo (CI)
	Pre- treatment	Post- treatment	Pre- treatment	Post- treatment	
Aortic PWV (m/s) n=22 (11:11)	13.5 (2.9)	10.6 (2.1)	11.6 (1.5)	11.5 (3.0)	-2.8 (-5.2, -0.3)
Brachial PWV (m/s) n=10 (4:6)	9.3 (1.9)	9.2 (1.6)	7.7 (2.1)	6.7 (1.7)	0.9 (-1.3, 3.1)
Seated AIX (%) n=20 (10:10)	29.8 (7.3)	30.4 (9.2)	23.4 (10.5)	20.2 (10.9)	3.8 (-2.6, 10.2)
Peripheral seated MAP (mmHg) n=23 (11:12)	116 (18)	111 (14)	115 (21)	113 (16)	-3.0 (-10.2, 4.3)
Peripheral supine MAP (mmHg) n=23 (11:12)	111 (17)	108 (18)	105 (19)	110 (20)	-8.1 (-16.7, 0.5)
Central seated MAP (mmHg) n=21 (9:12)	120 (19)	114 (15)	114 (24)	114 (19)	-5.0 (-11.8, 1.9)
Central supine MAP (mmHg) n=22 (10:12)	119 (18)	112 (20)	107 (21)	108 (25)	-7.2 (-17.2, 2.9)
Seated HR (bpm) n=22 (10:12)	77 (13)	75 (12)	73 (19)	78(21)	-6.7 (-12.2, - 1.2)
Supine HR (bpm) n=22 (10:12)	74 (12)	72 (8)	71 (16)	74 (20)	-5.0 (-11.2, 1.3)

† geometric mean

**Abbreviations-** **PWV:** pulse wave velocity; **AIX:** augmentation index; **MAP:** mean arterial pressure; **HR:** heart rate

**Figure 3.4 Change in Aortic PWV After Treatment with Simvastatin or Placebo in the Sub-group with Baseline Aortic PWV >10m/s**

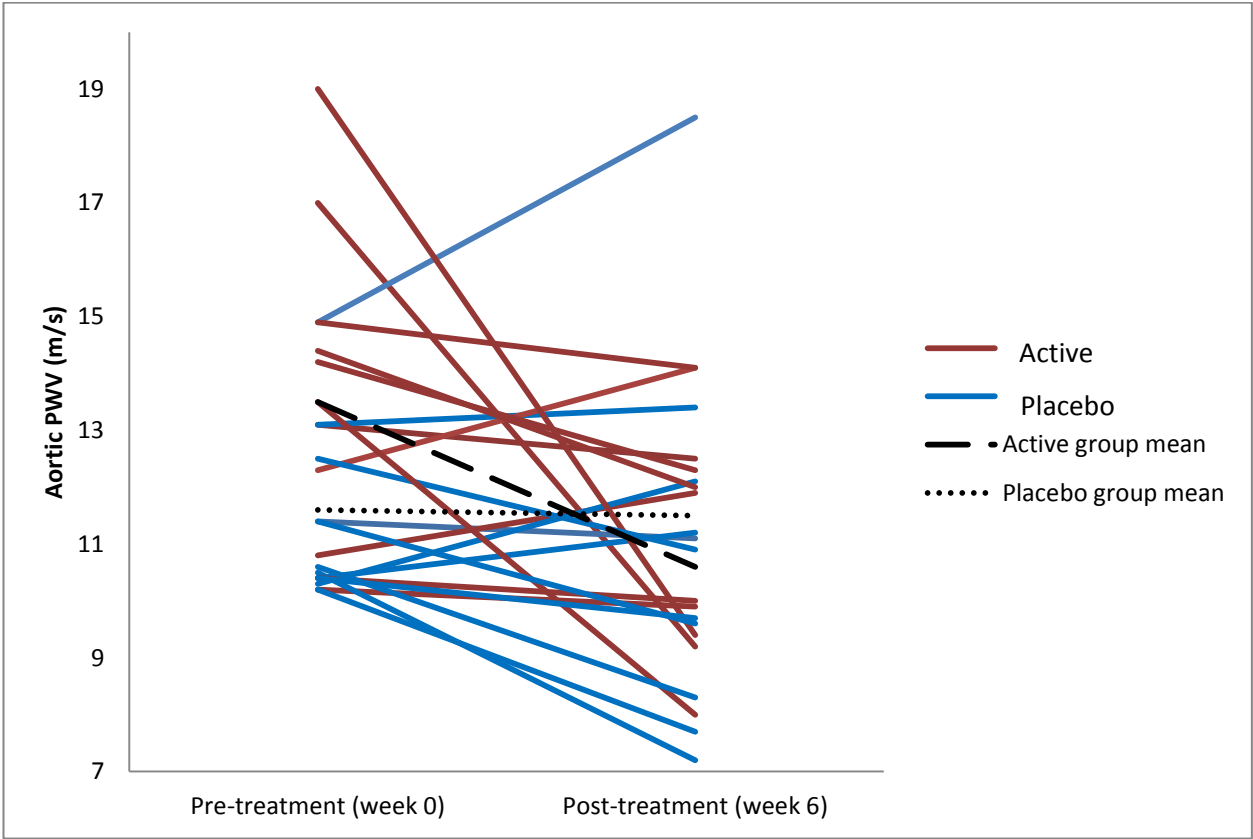


**Abbreviations – PWV:** pulse wave velocity

Mean change in aortic PWV (m/s) from baseline following 6 weeks treatment with active (simvastatin) or placebo in the sub-group with a baseline aortic PWV >10m/s.

Error bar = standard error

**Figure 3.5 Change in Aortic PWV for Individuals in the Subgroup with a Baseline Aortic PWV>10m/s**



Aortic PWV values (m/s) for each subject and mean values for active (simvastatin) and placebo subjects pre- and post-treatment, in the subgroup with a baseline aortic PWV >10m/s.

### **3.3.8      Side Effects**

Patient reported side effects were recorded, with adverse events such as musculoskeletal and respiratory being the most commonly reported. Musculoskeletal problems are commonly reported amongst statin users and were anticipated.

Side effects most commonly reported were respiratory events – including cough, shortness of breath and chest infections, and musculoskeletal – including muscle and joint aches, pain and cramps; less frequent were gastro-intestinal effects. There were more musculoskeletal side effects reported in the active group, as expected, with the other side effects comparable between the active and placebo group.

There were no severe adverse events and no mortality. The greatest proportion of subjects reported no side effects or adverse events at all. See Table 3.6.

**Table 3.6 Safety Issues Reported in the Total Study Group**

			Active	Placebo
Adverse Events	Musculoskeletal	Muscle cramps	2	0
		Muscle aches	5	2
		Joint aches	0	2
	Respiratory		4	3
	Gastrointestinal		1	1
Severe Adverse Events	Hospital admissions		0	0
	Death		0	0
Change in Liver Function Tests	Clinically relevant increase in ALT		0	0
	Clinically relevant increase in gamma GT		1	1
Clinically relevant increased CPK			0	1

**Abbreviations:** ALT: alanine aminotransferase; gamma GT: glutamyltransferase;  
CPK: creatine phosphokinase

### 3.4 Discussion

In this double blind, randomised controlled trial of 70 patients with COPD and without concurrent heart disease there was no significant difference in the change in aortic stiffness, as measured by PWV following 6 weeks, once daily, treatment with 20mg simvastatin compared to placebo; despite a reduction in total cholesterol of -1.2mmol/l in the active group. The active group had a change in aortic PWV of -1m/s post treatment, but as the placebo group had a drop of 0.3m/s the difference between groups was not significant. This was coupled with no significant change in other haemodynamic measurements including blood pressure.

The baseline results for subjects randomised to simvastatin were well matched to those in the placebo group. To date, this study is the only reported RCT of statins on aortic stiffness, or indeed any CV outcome, in patients with COPD. We used aortic PWV as the primary outcome measure because it is the gold standard measurement, has been shown to be an independent predictor of CV events (Laurent et al., 2001) and is responsive to change over a short period of time (Maki-Petaja et al., 2007). Retention in the study was good, with a drop-out rate of 9%. Whilst we measured Alx and brachial PWV these are not prognostic like aortic PWV.

The reported aortic PWV is recurrently lower in recently published studies (John et al., 2013, Dransfield et al., 2011) than original reports (Maclay et al., 2009, Sabit et al., 2007) on which this RCT was designed. This is despite aortic PWV measurements being recorded using similar devices. There are no obvious reasons why our PWV are lower than the original published results – baseline demographics are similar, with well-matched age, lung function and cholesterol levels. The

previously reported higher results are in a male population with lower blood pressure than described here (Maclay et al., 2009), and have a higher proportion of current smokers and pack year history (Sabit et al., 2007). Arguably, the lower than previously reported baseline aortic PWV that we observed could be due to the improvements in COPD care and medications over the intervening years. Our baseline aortic PWV values are comparable to other interventional studies. The active group had a baseline value of 9.9m/s compared to 10.1m/s (Dransfield et al., 2011) in a comparable COPD population; and 9.6m/s (Maki-Petaja et al., 2007) in patients with Rheumatoid arthritis. We have shown a decrease in aortic PWV from 9.9m/s pre-treatment to 8.9m/s post simvastatin treatment, compared to another interventional study using simvastatin that showed a decrease from 9.6m/s to 8.9m/s following treatment (Maki-Petaja et al., 2007), but again this is a rheumatoid arthritis population, so although it is a chronic inflammatory condition, it is not a COPD study.

The decrease in aortic PWV in the active group was to a result within the expected range for this population, but this drop was not enough to be significant compared to the placebo group. This remained after adjusting for age and sex.

Multiple linear regression showed seated MAP and age were predictors of baseline aortic PWV as one would expect. This is reassuring as age (McEniery et al., 2005) and MAP are known to be the main contributing factors to aortic PWV.

No other haemodynamic variables had a significant change post-treatment in the active compared to placebo group. As expected there was no change in BP, which was an important finding because lowering BP is a significant variable for aortic



PWV, and therefore any changes in aortic PWV ideally should be independent of BP changes. Statins have been reported to lower BP through increased NO bioavailability and improved arterial compliance, with meta-regression analysis showing no difference among different statin types (Briasoulis et al., 2013), however, we did not find such an effect in our study group.

Surprisingly, the central seated pulse pressure did not follow the same trend and was advantageous in the placebo group. Pulse pressure (PP) has previously been used as a surrogate marker of arterial stiffness and a predictor of CV mortality (Norata et al., 2013), where a 10mmHg increase in PP was associated with an increase in all-cause mortality of 11% (Abernethy et al., 1986, Biere-Rafi et al., 2013). We cannot explain this finding, but it may be a result of multiple t-tests, and of note it was not repeated in the central supine pulse pressure.

In the sub-group of those with a baseline aortic PWV >10m/s there was a significant difference in the change in aortic PWV between the active and placebo group post treatment, which remained after adjusting for age, sex, total cholesterol levels and change in MAP; indicating that intervention with statin therapy maybe better directed at those with a higher baseline. Importantly, there were no parallel changes in other haemodynamic variables post-treatment in the active compared to placebo sub-group.

There was no obvious way of identifying who would have the higher baseline values of aortic PWV, as shown by the similar baseline characteristic between the main group and sub-group with aortic PWV >10m/s. The total group and sub-group with a baseline aortic PWV >10m/s had matched baseline and demographic data with

the exception of systolic blood pressure and MAP being higher in the sub-group compared to the main group.

Identifying suitable subjects to take part in the study was a challenge; where 165 subjects were screened to meet the recruitment target, with the main reasons for failing screening being hypercholesterolaemia and spirometry/reversibility results. The number of inclusion and exclusion criteria made the study population very specific, and therefore may reduce the generalizability of the findings to clinical settings; however, this was a proof of principle study. One could question how representative our sample was, but we were keen from the outset to establish the role of statins in a population where they are not currently indicated. If we had taken all COPD subjects it would then be questioned whether any reported benefit was simply because our population met the current licencing requirement for statin therapy.

There is increasing interest in using aortic PWV as an outcome measure, and to screen patients for high aortic PWV at baseline. Current interventional studies in patients with COPD are now opting to use aortic PWV as inclusion criteria, such as a study by GSK looking at aortic stiffness after 24 weeks fluticasone furoate/vilanterol intervention. We did not because it is not a routine clinical test. However, following the sub-group analysis showing a significant change in aortic PWV in the active group compared to the placebo group, it may have been worth considering this as an entry criteria. The exclusion criteria meant that comorbidities such as a history of CV disease, hypercholesterolaemia or diabetes mellitus which would result in an increased aortic PWV were not present. Currently, aortic PWV is not

measured as part of routine clinical care, but such central haemodynamic measures are welcomed by the FDA and undergoing review (Townsend et al., 2010, McEniery et al., 2014).

Describing 'normal' values for aortic PWV is contentious as there are not well defined normal ranges and the variety of devices and techniques that can record measurements adds to the variability. All pre- and post-treatment study measurements were made by one person in this trial to standardise technique and prevent operator variability. An aortic PWV cut-off of 12m/s was first proposed in the ESH/ESC 2007 guidelines for the management of arterial hypertension (Mancia et al., 2007) to determine increased aortic stiffness. Since then a 10m/s cut off in aortic PWV has been proposed to determine increased results (Van Bortel et al., 2012, Boutouyrie et al., 2010), so we used that for our sub-group analysis; and this has since been adopted by the latest ESH/ESC guidelines (Mancia et al., 2013). However, adding to the controversy an aortic PWV cut-off of 11m/s was suggested, and recent studies have also used this as the cut-off for determining high values (Phababpha et al., 2013).

We have considered why the active group did not have any significant change post-treatment compared to the placebo group. There are several possible explanations for the lack of response we saw following treatment including compliance, the duration of the study, lower baseline results as discussed and co-existent medication. Taking these in turn, the lack of change in results in the active group post-treatment compared to the placebo group is unlikely to be due to compliance, as on the whole compliance data was good.

In retrospect our sample size was too small to detect a significant change in aortic PWV after treatment. As the confidence interval (CI) of mean difference between groups includes -1, our findings do not rule out a potential benefit that could be seen in an adequately powered study.

The drop in aortic PWV reported in the placebo group could be attributed to the 'placebo effect', the apparent benefit observed during study participation, as first described by Beecher in 1955 (Beecher, 1955), or as a result of spontaneous improvement, fluctuation of symptoms or regression to the mean – all of which have been reported as false impressions of the placebo effect (Kienle and Kiene, 1997). It has been stated that altering the therapeutic environment may significantly contribute to reducing clinical symptoms (Mayberg et al., 2002) and therefore have impacted upon the clinical measures.

The onset of action of the treatment drugs should not be a factor as a significant decrease in cholesterol levels shows medication was taken, and effective. The 6 week duration of the intervention period should have been long enough to see an effect, as previous studies in other chronic inflammatory conditions have shown a reduction in aortic PWV in the same timeframe (Maki-Petaja et al., 2007), and we were able to demonstrate the duration was sufficient to decrease cholesterol.

We used a relatively low dose of simvastatin 20mg od and we cannot exclude the possibility that a higher dose of 40mg od may have shown a better response, however, in a rheumatoid arthritis population it was sufficient dose and duration to exhibit a benefit on aortic PWV (Maki-Petaja et al., 2007). We selected a dose of 20mg simvastatin because with a dose of 40mg there are higher risks of

musculoskeletal problems and other side effects. From a patient perspective there does not appear to be any undue safety concerns in using statins at this dose in a COPD population.

One placebo controlled RCT with the primary endpoint of aortic PWV has now been reported; where 249 patients with COPD were randomised to either fluticasone propionate/salmeterol combination inhaler twice daily or placebo, a reduction in aortic PWV was not seen. However, post hoc analysis found a response in the subgroup of those patients with higher baseline aortic PWV (Dransfield et al., 2011). We excluded medications which were likely to affect aortic PWV such as oral steroids but were not able to control for combination inhalers, however, no patient knowingly started a new treatment during the six weeks.

Aortic stiffness can be affected by both structural and functional aspects of the conduit arteries and vascular beds. Systemic inflammation and endothelial dysfunction have been proposed as potential explanations for the increased aortic stiffness and CV risk in subjects with COPD (Mills et al., 2008). Endothelial impairment has been suggested as a cause of increased arterial stiffness, as seen in patients with COPD; however, a recent study suggests that increased arterial stiffness is not a result of endothelial dysfunction (Maclay et al., 2009). Structural aspects are less modifiable and include things like calcification, which often arises as a result of untreated functional aspects such as hypertension. Calcification leads to decreased compliance and structural changes in the vessel wall. Both aortic stiffness and calcification increases with age (Mills et al., 2008). By treating potentially modifiable factors functional and structural changes can be prevented

and we may be able to lower aortic PWV. Early anti-inflammatory intervention may help to modify the functional changes and therefore delay any structural changes.

All of our subjects had a significant smoking history, as associated with COPD. Smoking is a common, well described risk factor for both COPD and CV disease, however patients with COPD have increased risk of CV disease over and above the effects of smoking (Sin and Man, 2003) – the cause of which is not entirely understood, but may be caused by systemic inflammation. In moderate-severe COPD subjects this low-grade inflammation has been associated with the risk of cardiac injury (Sin and Man, 2003).

#### **3.4.1      Limitations**

Although the planned sample size was reached, in light of the lower baseline aortic PWV and wider SD compared to what the power calculation was based on, it was underpowered; and a possible cause of the absence of any statistically significant changes in primary and secondary outcome measures between active and placebo group. We now know we would need 177 patients in each arm of the study for 90% power to detect a -1m/s drop in aortic PWV. The study was powered to detect a 1m/s decrease in the active group, but the power calculation did not account for changes in the placebo group; therefore the 0.3m/s drop we reported in the placebo group was not pre-considered; however, this is in part accounted for by looking at the change between groups. The supportive findings of simvastatin in patients with COPD with a higher baseline aortic PWV are encouraging but there was some disparity in baseline haemodynamic measures between the active and placebo groups.

### **3.4.2      Future Implications**

This was a pilot study to investigate feasibility for future studies. Any intervention that may reduce the risk of CV disease in COPD requires careful evaluation. Future studies could take two directions. One direction would be a larger and more pragmatic study looking at hard end-points such as CV morbidity and/or mortality, although these are costly. Alternatively, to have aortic PWV as the primary endpoint, in a larger multi-centre study recruiting those subjects with a high baseline aortic PWV.

There were difficulties in recruitment and these should be considered in the planning of future studies. Intervention with statin therapy appears better directed at those with an aortic PWV  $>10\text{m/s}$ . Aortic PWV is not routinely performed in the clinical setting, therefore further investigation to see if it is practical to use as a screening tool to risk stratify and identify those with increased aortic PWV, a marker of increased CV risk, hence would benefit from statin treatment or further investigation and intervention is required.

### **3.4.3      Conclusion**

Despite a significant reduction in total cholesterol there was no significant improvement in aortic PWV in patients with COPD taking simvastatin 20mg od compared to placebo over 6 weeks. In the subgroup with a baseline aortic PWV value of  $>10\text{m/s}$  we did however show a significant change in aortic PWV post-treatment in the active compared to placebo group. Therefore, subjects with a higher baseline aortic PWV are likely to have more benefit from intervention.

Statins appear safe to use in this population and this proof of principle study supports investigating this further and can be used to assist the design and methodology of such a study.



## **Chapter 4**

### **The Inflammatory Effects of Simvastatin in Patients with COPD:**

**A double blind, randomised  
placebo controlled trial**

## **4 The Inflammatory Effects of Simvastatin in Patients**

### **with COPD: A double blind, randomised placebo**

### **controlled trial**

#### **4.1 Introduction**

Current therapies for patients with COPD, which are routinely inhaled bronchodilators to help with airway obstruction, are largely symptom based or exacerbation related. The only current disease modifying intervention is smoking cessation (Anthonisen et al., 1994, Decramer et al., 2013). Whilst there are new drugs for COPD in the pipeline, it is a long process which can take years to reach the patient; and it has proved difficult to identify new agents which are safe and effective (Barnes, 2013). Therefore, exploring old drugs for new indications is advantageous.

COPD is defined by its airflow obstruction but there is a chronic persisting inflammatory process, reflected in increased airway and circulating inflammatory markers (McCurdy et al., 2011, Miller et al., 2013, Pinto-Plata et al., 2006). Attenuating inflammation has the potential to alter the disease course (Sin et al., 2004) and the systemic inflammatory process is thought to contribute to the comorbidities of COPD (Gan et al., 2004).

Statins have been proposed as a potential respiratory disease modifier in review articles since as far back as 2006 (Hothersall et al., 2006, Dobler et al., 2009, Young et al., 2009). Previous studies have suggested statins as a potential therapy to

improve lung function due to their anti-inflammatory and antioxidant effects (Alexeeff et al., 2007, Keddissi et al., 2007). Statins have also been shown to decrease specific inflammatory markers such as IL-6 (Inoue et al., 2000), CRP (Arnaud et al., 2005) and MMP levels (Bellosta et al., 1998), in other conditions, but all clinically relevant mediators in COPD.

Spirometry, specifically FEV<sub>1</sub>, is used as one marker of COPD severity; and has been shown to be predictive of both CV and total mortality (Hole et al., 1996). The rate of decline of lung function is associated with adverse outcomes, such as mortality and COPD related hospital admissions (Mannino and Davis, 2006). There have been long standing discussions regarding statins and their potential to reduce the rate of decline in lung function results in both current and ex-smokers with abnormal baseline spirometry (Keddissi et al., 2007), and a slower rate of decline in lung function has been reported in those concurrently taking statins (Alexeeff et al., 2007).

There are several anti-inflammatory agents used in COPD. Inhaled corticosteroids (ICS) have an anti-inflammatory effect in patients with COPD, where those using ICS had lower CRP levels than those not using ICS (Pinto-Plata et al., 2006). In a RCT studying patients (non-COPD) admitted to hospital with acute coronary syndromes, using statins to lower CRP <2mg/l were associated with improvements in event free survival (Ridker et al., 2005a).

Nitric oxide (NO) is a gaseous signalling molecule produced by three isoenzymes of NO synthase (NOS) found in airway epithelial and circulating endothelial cells, as well as trafficking inflammatory cells in large and peripheral airways (Barnes et al.,

2010). Endothelial nitric oxide synthase is reduced in the peripheral lung of subjects with severe stages of COPD due to the emphysematous destruction of alveolar walls (Brindicci et al., 2010).

**Hypothesis:**

**Simvastatin 20mg taken once daily for 6 weeks will reduce airway (sputum and exhaled nitric oxide) and circulating (blood) inflammatory markers compared to the placebo group in patients with COPD.**

## **4.2 Methods**

This study was a double-blind, placebo controlled RCT, see methods p63. Clinical trials identifier: NCT01151306.

### **4.2.1 Recruitment**

Subjects with COPD were recruited into the study as described in methods chapter (chapter 2 p68) following detailed inclusion and exclusion criteria (methods 2.1.2). The inclusion and exclusion criteria were more pertinent to the cardiovascular primary outcome measures.

Following consent and screening as outlined previously, methods 2.1.5 page 73, eligible patients were randomised methods 2.1.9 page 75.

### **4.2.2 Visit 1**

During visit one the baseline data was collected. Tests performed pertinent to this chapter are post-bronchodilator spirometry - FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC ratio, FEV<sub>1</sub> % predicted and FVC % predicted (methods 2.2.3); FeNO - measured by steady exhalations at varying flow rates – 10, 30, 50, 100 and 200ml/s; see methods 2.2.4. The alveolar slope and bronchial intercept were also recorded. Induced sputum - performed by nebulising a hypertonic saline solution (methods 2.2.5) and sputum cell counts (see methods 2.2.6); venepuncture – safety bloods (LFT and CPK), lipid profile (see methods 2.2.7.a), circulating MMP-9 and hs-CRP (see methods 2.2.7.c.i and 2.2.7.c.ii). Body composition (see methods 2.2.13) and functional assessment was performed using dominant handgrip strength (methods 2.2.14) and 6 minute walking distance (6MWD) (methods 2.2.9).

#### **4.2.3      Treatment Phase**

Telephone calls were made to each participant two and four weeks post visit one, to check compliance and for any problems.

#### **4.2.4      Visit 2**

Post-treatment results were recorded as per visit one. See methods p77. Compliance was checked and recorded. We checked for any clinically relevant increases in gamma GT, ALT and CPK as previously described.

#### **4.2.5      Data Analysis**

Data was analysed using Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 21.0. A  $p < 0.05$  was considered significant. The normality of the data was checked graphically. Graphical interpretation was made using frequency distribution histograms and Q-Q plots. Positively skewed data was  $\log_{10}$  transformed to normalise the data – sputum neutrophils and macrophages, hs-CRP and FeNO50, alveolar gradient and bronchial intercept - in order to perform parametric analysis. Unless otherwise specified, arithmetic mean and standard deviation was used to show the measure of central tendency.

**Analysis:** The difference in the mean change for the active and placebo arms was analysed using independent t-test and the mean and 95% confidence intervals presented. This was done for the following: FEV<sub>1</sub>, FVC, total cholesterol, HDL and LDL cholesterol, 6MWD, sputum neutrophils, blood neutrophils, FeNO50, intercept and gradient, and serum biomarkers including total MMP-9 and hs-CRP. The difference in change in SGRQ scores, FFM and BMI were also analysed.

Sputum neutrophilia was defined as  $\geq 60\%$  sputum neutrophils (Spanevello et al., 2000). Sputum eosinophilia was defined as  $\geq 3\%$  (Pavord et al., 1999).

A sub-group analysis was performed on subjects with a baseline circulating hs-CRP value of  $>3\text{mg/l}$ , as per the Rotterdam study (Lahousse et al., 2013), to look at the change in hs-CRP, sputum neutrophils and macrophages, and FeNO50, bronchial intercept and alveolar gradient post-treatment in the active group compared to the placebo group.

## 4.3 Results

### 4.3.1 Demographics

64 subjects completed the study out of the 70 randomised and starting the study medication, see consort diagram Figure 3.1 p100. No follow on data is available for those who dropped out of the study, see p105.

Baseline data for those on active treatment are presented alongside the placebo group, see Table 3.2 p106. Groups were well matched for age, sex, smoking pack years, lung function and baseline cholesterol levels.

### 4.3.2 Missing Data

Sputum results are not available in all subjects. Baseline sputum results are only available for n=27 – active n=11, placebo n=16; and matched post-treatment data is available for n=20 – active n=8, placebo n=12.

At baseline, of the 43 subjects without results 23 could not perform the induction procedure because they had a post-bronchodilator FEV<sub>1</sub> of <50% which is a contraindication to performing the procedure. Nine subjects were unable to produce a sputum sample following the induction process, 5 produced a sample but the slide was uncountable, therefore results could not be obtained and 6 declined the sputum induction procedure. Reasons for the slides being uncountable and therefore unable to provide results from the given sputum sample included squamous contamination, damaged cells causing poor viability and the sample being saliva rather than sputum.



Exhaled nitric oxide levels could not be performed in every subject at both visits as there were recurrent technical issues and some patients struggled with the test technique. Matched post-treatment results for FeNO50 were available in n=36 with 17 in the active and 19 in the placebo group.

### **4.3.3      Airway Measures**

#### *4.3.3.a Sputum*

There were no significant changes in the difference in change in sputum differential cell counts in neutrophils or macrophages, where both were measured as absolute number of cells and percentage of the total cells, in the active group compared to the placebo group. See Table 4.1; and Figure 4.1.

Of the 27 subjects at baseline, 19 (70%) had baseline sputum neutrophilia - active n=9, placebo n=10. A further 3 people had sputum neutrophils  $\geq 60\%$  but also had sputum eosinophils  $\geq 3\%$ . There were 2 subjects which had baseline sputum eosinophilia only.

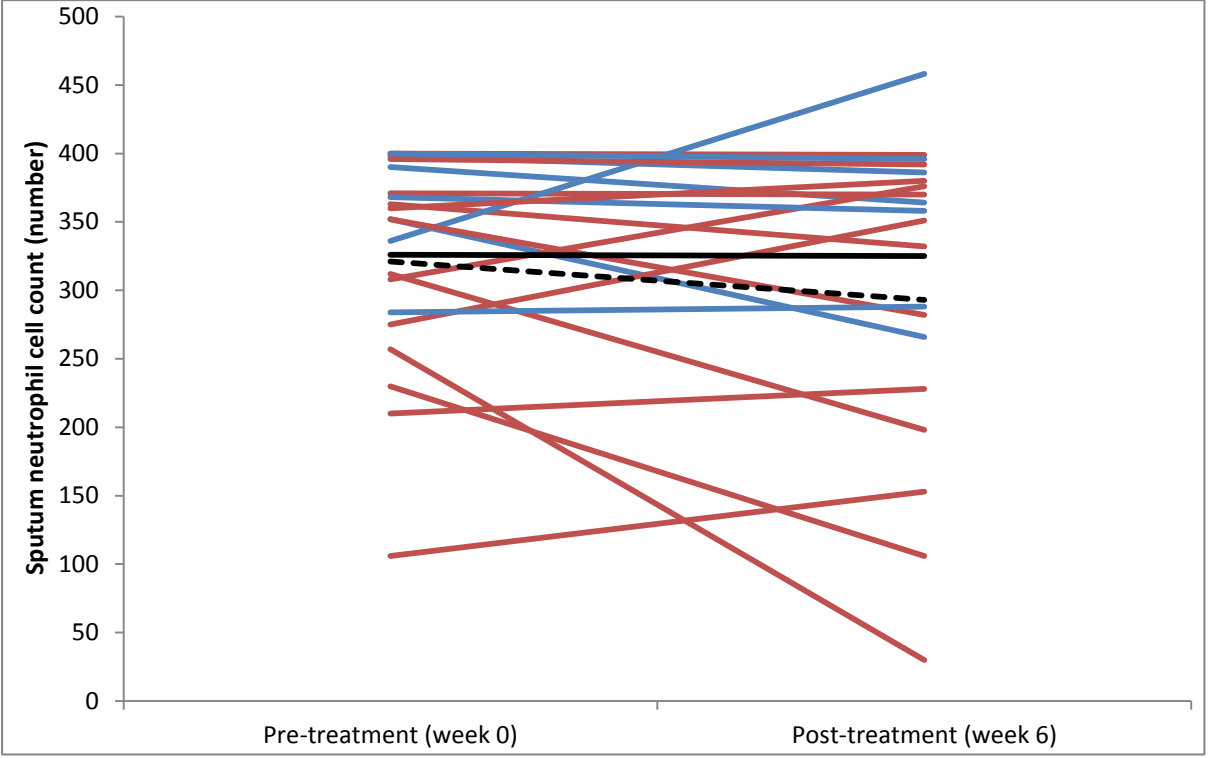
Of those with sputum neutrophilia (+/- eosinophilia) there were paired pre- and post-treatment results in n=19 (active n=8, placebo n=11). There was not any significant change in sputum neutrophils post treatment in the active compared to the placebo group.

**Table 4.1 Physiological, Airway and Inflammation Markers in the Active (simvastatin) and Placebo Groups**

Mean (SD)  Unless otherwise specified n=active: placebo	Active group			Placebo group			Mean difference Δ active versus Δ placebo (CI)
	Pre - treatment	Post - treatment	Post –Pre Mean 'Δ'	Pre - treatment	Post - treatment	Post-Pre Mean 'Δ'	
FEV <sub>1</sub> (L) n=31:33	1.59 (0.6)	1.59 (0.6)	0.0	1.41 (0.5)	1.43 (0.6)	0.0	0 (-0.07, 0.05)
FVC (L) n=31:33	3.40 (0.9)	3.3 (0.9)	-0.1	3.06 (1.0)	3.07 (1.0)	0.0	-0.1 (-0.2, 0.2)
Sputum neutrophils (%)† n=8:12	75.2 (1.5)	75.3 (1.4)	-1.9	77.5 (1.3)	61.4 (2.2)	-6.3	4.4 (-12.0, 20.8)
Actual sputum neutrophils (number)† n= 8:12	306 (2)	310 (1)	-2.8	314 (1.2)	246 (2)	-27.8	25.0 (-50.1, 101.0)
Sputum macrophages (%)† n=8:12	12.0 (1.6)	6.6 (4.8)	1.7	8.8 (4.1)	10.5 (3.7)	5.7	-4.0(-16.4, 8.4)
Actual sputum macrophages (number)† n= 8:12	34 (2)	27 (5)	7.3	32 (4)	42 (4)	20.4	-13.2 (-63.1, 36.8)
FeNO 50mls (ppm) † n=17:19	23.6 (1.9)	21.8 (1.9)	-2.2	14.5 (1.9)	17.8 (2.1)	6.1	-8.2 (-19.1, 2.6)
FeNO intercept † n=16:19	34.4 (2.5)	36.9 (2.5)	-0.1	27.7 (2.1)	31.1 (2.2)	4.4	-4.5 (-25.3, 16.4)
FeNO gradient † n=16:19	8.9 (2.5)	8.1 (2.5)	-2.0	4.8 (2.1)	5.9 (3.2)	3.8	-5.9 (-13.5, 1.7)
Circulating MMP-9 (ng/ml) n=31:32	53.1 (16.3)	50.4 (16.8)	-2.9	60.4 (17.2)	56.1 (17.8)	-3.1	0.2 (-10.8, 11.2)
Circulating hs-CRP(mg/l) † n=31:32	3.5 (3.1)	2.9 (3.9)	0.7	3.0 (3.0)	3.0 (2.9)	0.2	0.5 (-3.2, 4.1)
Dominant hand grip strength (kg) n=31:33	35.3 (9.2)	35.3 (8.5)	0.1	32.2 (8.3)	33.0 (8.4)	0.9	-0.8 (-2.6, 0.9)
6MWD (m) n=30:31	343 (75)	339 (84)	-3.6	332 (76)	338 (88)	5.1	-8.7(-23.6,6.2)
SGRQ total n=31:33	36.8 (15.4)	38.1(16.3)	1.3	35.4 (18.7)	34.3(19.9)	-1.1	2.4 (-1.1, 6.0)
FFMI n=27:29	18.7 (2.5)	18.7 (2.4)	-0.6	18.6 (2.8)	18.3 (2.7)	-0.6	0 (-0.9, 0.9)
BMI (kg/m <sup>2</sup> ) n=28:30	26.7 (6.7)	26.7 (6.9)	0.1	26.6 (6.8)	26.8 (6.8)	0.1	0 (-0.2, 0.3)

**Key:**†geometric mean **Abbreviations:** **FEV<sub>1</sub>**: forced expired volume in one second; **FVC**: forced vital capacity; **FeNO**: fraction of expired nitric oxide; **MMP-9**: matrix metalloprotease-9; **CRP**: C-reactive protein; **6MWD**: six minute walking distance; **SGRQ**: St George's Respiratory Questionnaire; **FFMI**: fat free mass index; **BMI**: body mass index

**Figure 4.1 Individual Sputum Neutrophil Counts Pre- and Post-treatment with Active (simvastatin) or Placebo**



**Key:**

- Active group
- Placebo group
- Active Mean
- Placebo Mean

#### *4.3.3.b Exhaled Nitric Oxide*

There were no significant differences in the change of any FeNO measures – FeNO<sub>50</sub> (n=36), alveolar gradient (n=35) or bronchial intercept (n=35) post-treatment in the active group compared to the placebo group. Baseline mean FeNO<sub>50</sub> was 23.6ppm for the active group (n=17) and 14.5 for placebo group (n=19).

### **4.3.4 Systemic/Circulating Inflammatory Mediators**

#### *4.3.4.a Circulating MMP-9*

There were no significant differences in the change in MMP-9 measurements pre- and post-treatment between the active group compared to the placebo group. See Table 4.1.

#### *4.3.4.b Hs-CRP*

There were no significant differences in the change in hs-CRP measurements pre- and post-treatment between the active group compared to the placebo group, see Table 4.1. The mean differences presented in Table 4.1 appear to show an increase in hs-CRP result post treatment; however, this has just arisen through using mean values; because one result could skew the mean, but the mean differences are differences within an individual and then summed, and not the differences of the whole population.

### **4.3.5 Sub-group Analysis of hs-CRP >3mg/l**

For the whole group, the mean hs-CRP was greater than control data in other studies (Pinto-Plata et al., 2006), but there was a range. The CRP >3mg/l sub-group (n=33: 16 active, 17placebo) was comparable to the main group, with similar age, proportion of males, smoking pack years, lung function and cholesterol levels. The

CRP >3mg/l sub-group had a higher smoking pack year history (48 pack years) than the main group (40 pack years).

The active and placebo groups of the CRP >3mg/l sub-group were well matched for demographic data and sputum and CRP inflammatory markers (see Table 4.2); however, there was a significant difference in baseline FeNO50 and bronchial intercept,  $p<0.001$ ,  $p=0.03$  respectively between the active and placebo arms, where values were higher in the active arm, as per findings in the total study group.

In the sub-group analysis of subjects with a baseline circulating CRP value of >3mg/l (Lahousse et al., 2013), there was no significant difference in change post-treatment in hs-CRP – mean difference and 95%CI 0.26 (-6.6, 7.1)mg/l, MMP-9 -1.2 (-18.3, 15.8)ng/ml, sputum neutrophils 39.6 (-45.2, 124.5)n or macrophages -34.5 (-106.9, 37.9)n; or FeNO50 -2.6 (-20.6, 15.4)ppm, bronchial intercept -4.7 (-49.5, 40.1) or alveolar gradient -2.6 (-11.5, 6.3) in the active group compared to the placebo group.

**Table 4.2 Demographics and Baseline Inflammatory Markers in Sub-group with a hs-CRP>3mg/l**

<b>Mean (SD) Unless otherwise specified (active:placebo) (t-test unless otherwise stated)</b>	<b>Active n=16</b>	<b>Placebo n=17</b>
<b>Age (years) Median (range) (16:17)</b>	65 (50-77)	63 (51-74)
<b>Sex Male n (%) (16:17) *</b>	13 (82)	10 (59)
<b>Smoking pack years (16:17)</b>	47 (22)	51 (21)
<b>FEV<sub>1</sub> (l) (16:17)</b>	1.57 (0.50)	1.28 (0.35)
<b>FEV<sub>1</sub> %predicted (16:17)</b>	55 (12)	49 (12)
<b>FEV<sub>1</sub>/FVC (16:17)</b>	48 (12)	46 (12)
<b>Total cholesterol (mmol/L) (16:17)</b>	5.4 (0.8)	5.2 (0.8)
<b>hs-CRP (mg/l)† (16:17)</b>	8.2 (2.2)	7.0 (1.7)
<b>MMP-9 (ng/ml) (16:17)</b>	57.3 (15.2)	66.0 (17.2)
<b>Sputum neutrophils (counts)† (6:9)</b>	288 (2)	313 (1)
<b>FeNO50 (ppm)† (13:15)</b>	25.5 (1.7)	9.4 (1.9)
<b>FeNO Bronchial intercept† (10:14)</b>	39.1 (3.1)	13.3 (4.0)
<b>FeNO Alveolar gradient† (10:14)</b>	7.0 (3.3)	3.6 (1.9)

† geometric mean \* chi-squared test

**Abbreviations:** FEV<sub>1</sub>: forced expired volume in one second; FVC: forced vital capacity; hs-CRP: high sensitivity C-reactive protein; MMP-9: matrix metalloprotease-9; FeNO: fraction of expired nitric oxide

#### **4.3.6      Other measurements**

There was a significant decrease in total and LDL-cholesterol levels in the active group compared to placebo group post treatment ( $p < 0.001$  respectively) as reported in chapter 3.

There was no significant difference in change between the active compared to placebo arm following treatment in BMI, FFMI, SGRQ, dominant hand grip strength, 6 MWD or lung function as measured by  $FEV_1$  and  $FEV_1\%$  predicted, see Table 4.1.

## 4.4 Discussion

In this double blind, randomised, placebo controlled trial of 70 patients with COPD there was no significant difference in change in any airway or circulating inflammatory markers between the active statin group compared to the placebo group over 6 weeks. In addition, there were no changes in spirometric measures, or functional tests as measured by dominant handgrip strength and 6MWD. We did show a significant reduction in total cholesterol levels in the active statin group compared to placebo group post-treatment.

### 4.4.1 Airway Inflammatory Markers

Patients with COPD have been shown to have increased airway inflammation compared to non-atopic healthy subjects, irrespective of whether they are a current smoker or not (Rutgers et al., 2000). Our baseline sputum neutrophils results were comparable to those that Rutgers *et al.* reported where sputum neutrophils in patients with COPD had a median (range) of 77 (29-94)%.

Although 2 subjects were found to have solely eosinophilic sputum at baseline, we had excluded anyone reporting a previous diagnosis of asthma, and performed reversibility testing at the screening visit to try to eliminate any significant bronchial hyper-responsiveness; we did not have sputum cell counts as an inclusion/exclusion criteria. A high proportion of our subjects had sputum neutrophilia, characteristic of patients with COPD. Absolute neutrophil numbers are variable over time and so neutrophils as a percentage of total cells are more informative in general for COPD subjects (Singh et al., 2010); however for an RCT absolute cell number may have merit and so both absolute and percentage of the total were recorded.



The use of FeNO in COPD is a contentious issue as it is an unanswered area with many outstanding research questions and ongoing debate. A cautious approach to nitric oxide result interpretation is required as it is not as well described in COPD populations as in asthma. We performed FeNO at multiple flow rates as this has been reported by Lehtimäki *et al.* to differentiate between alveolar and bronchial NO production and therefore allow large and small airway inflammation to be assessed in asthmatics, patients with alveolitis and healthy controls (Lehtimäki *et al.*, 2001). The five-flow method of measuring FeNO has been reported in patients with COPD and controls with no significant within- or between-day variability and that data from five-flow rates was more reliable than from fewer flow rates (Roy *et al.*, 2007).

A recent study looking at five-flow FeNO levels in COPD sub groups did not find any differences in FeNO measurements between their pre-defined sub-groups - severe emphysema, chronic bronchitis, frequent exacerbations, loss of lean body mass and low fat-free mass index; and the usefulness of FeNO in patients with COPD is still questioned (Bazeghi *et al.*, 2011). FeNO results are often reported as normal (Clini *et al.*, 2000) or increased (Beg *et al.*, 2009) with single flow measurements in patients with stable COPD despite significant airflow limitation hence it is a controversial measure. This could be contributable to a number of factors including the heterogeneity of the disease, smoking status or the use of ICS (Ansarin *et al.*, 2001, Barnes *et al.*, 2010). The factors contributing to increased FeNO levels in asthma are thought to be different to the factors influencing FeNO results in COPD (Ansarin *et al.*, 2001), this therefore, may be why the tests have currently been

shown to be more useful in asthma compared to COPD. The use of ICS has been reported to suppress FeNO (Barnes et al., 2010, Jones et al., 2002), although this is not universal (Ansarin et al., 2001).

Smoking status has been shown to influence FeNO results, and may in part explain why the evidence for its use in COPD varies so widely. Both current and past smoking has been shown to reduce FeNO results compared to never smoker controls (Malinovschi et al., 2006). Cigarette smoking significantly reduces FeNO results due to the down regulation of inducible nitric oxide synthase (iNOS) (Barnes et al., 2010, Kharitonov et al., 1995). Subjects were asked to refrain from smoking for 6 hours prior to their visit in an attempt to reduce any acute smoking related effects on results and to standardise methodology.

We were underpowered to detect any changes in FeNO and sputum results as these tests could only be performed in a sub-set of patients due to test technique, contra-indications to performing the test and technical problems with the machines.

#### **4.4.2      Circulating Inflammatory Markers**

It is recognised that “inflammation” covers a wide range of markers and we acknowledge that we have chosen specific markers to measure and analyse in this proof of principle study. The alternative would have been to take a blanket approach and test for many more markers, but we would then be at risk of finding a significant change as a result of multiple endpoints. Circulating inflammatory markers such as hs-CRP (Pinto-Plata et al., 2006) and MMP-9 (Bolton et al., 2009b) have been reported to be elevated in patients with COPD, with a potential

pathological influence, and these markers have also been shown to be modulated following statin treatment in other diseases (Bellosta et al., 1998, Ridker et al., 2009).

The main marker of systemic inflammation is hs-CRP which has been associated with increased cardiac injury (Sin and Man, 2003) and is available in all of the 70 baseline subjects and paired results in 63 subjects. CRP is an acute phase protein produced via stimulation of IL-6 released after vascular damage. It has been reported that CRP could affect vasomotor endothelial function through inhibition of endothelial nitric oxide (NO) synthase, and therefore inhibit production of NO (MacLay and MacNee, 2013). Hs-CRP results did not show any significant change in either the active or placebo group post-treatment.

Baseline inflammation in patients with COPD, as determined by CRP levels, has been shown to influence the benefit seen following statin therapy, where a significant drop in CV mortality was seen following statin therapy for greater than 2 years (Lahousse et al., 2013). A randomised control trial looking at hs-CRP levels in patients with stable COPD, using a different statin – pravastatin 40mg daily, and for a much longer duration (6 months) reported baseline results comparable to the baseline results we found with active and placebo hs-CRP of 3.94 and 4.06mg/l respectively (Lee et al., 2008), however, despite statin therapy a proportion of patients with COPD have persistently elevated hs-CRP levels (Lee et al., 2008, Ridker et al., 2005b). In comparison, Maki-Petaja *et al.* studied a rheumatoid arthritis population with a baseline serum CRP >6mg/l and found a significant reduction in CRP levels post 6 weeks treatment with simvastatin 20mg (Maki-Petaja et al., 2007).

A pravastatin 40mg daily dose is comparable to a simvastatin 20mg daily dose (Mehra et al., 2002, Keogh et al., 2000).

CRP has been used to grade the risk for future CV events with a CRP<1mg/l being low risk, 1-3mg/l moderate risk and >3mg/l high risk (Pinto-Plata et al., 2006, Ridker et al., 2002). A sub-group analysis was performed on patients with a baseline circulating hs-CRP value of >3mg/l, chosen because it has been identified as the high risk group and is a well published cut-off point in both general population studies (Ridker et al., 2002) and in COPD populations (Lahousse et al., 2013). When a high hs-CRP value is defined as >3mg/l, 51% of our study population would have been in the high risk group with 17/36 of those being in the active group and 19/36 being in the placebo group; but still we could not detect a change in any of our inflammatory markers following statin treatment. This is comparable to the 60% of people being in the high risk group reported by Pinto-Plata *et al.* in a cross-sectional study of patients with COPD and current and never smoker controls (Pinto-Plata et al., 2006).

Matrix metalloproteinases (MMPs) are thought to play a role in the pathology of COPD including tissue remodelling and repair (Haq et al., 2010). It has recently been reported that MMP-12, which is produced by macrophages and is found on chromosome 11q22.3, was a modifier of COPD disease severity (Haq et al., 2010) and it has a positive association with lung function in adults who either smoke or are at risk of COPD (Hunninghake et al., 2009). MMP-9 has been shown to have a role in the pathogenesis of emphysema and to act outside the lung in the development of arterial stiffness and atherosclerosis (Maclay and MacNee, 2013).

MMP-9 has been associated with increased arterial stiffness in the general population (Yasmin et al., 2005) and features of elastin degradation in the skin of patients with COPD (Maclay et al., 2012).

MMP-9 has been reported to be elevated in patients with COPD compared to controls in a matched case control study (Pinto-Plata et al., 2007). MMP-9 had a strong association with lung function, as measured by FEV<sub>1</sub> and TLco in patients with severe COPD and matched controls (Pinto-Plata et al., 2007). Our baseline MMP-9 results are not consistent with previously published findings, however, discrepancies between whether total, pro or active MMP-9 or MMP-9 activity was measured is often hard to determine from reported studies. Our baseline MMP-9 result for the total group was 61.4(16.1)ng/ml, which is lower than previously reported results of 248.2 ±101.0ng/ml in patients with COPD despite being of the same nature and similarly being reported as total (pro and active) MMP concentration, and measured using the same kit (Brajer et al., 2008). Circulating MMP-9 was shown to be elevated in patients with COPD compared to controls, with MMP-9 values of 38.5 (2.2)ng/ml reported, which is closer to our reported findings, however pro-MMP-9 was measured using a different ELISA kit (Bolton et al., 2009b).

Fibrinogen is an emerging inflammatory mediator with prognostic value in patients with COPD (Gan et al., 2004, Dahl et al., 2001), which has been highlighted more recently. Fibrinogen is elevated in stable subjects who have exacerbations of COPD, increases further during exacerbation presence, and may contribute to the increased CV morbidity and mortality (Wedzicha et al., 2000). It has been identified

as a potentially useful biomarker to identify those patients at risk of COPD in the adult Danish general population (Dahl et al., 2001), who have increased likelihood of poor health and may help to determine those with a better chance of responding to specific treatments.

#### **4.4.3      Airway and Functional Assessment**

It is perhaps not surprising that there was no change in spirometry or functional assessment given the 6 week time frame of the study. We did not expect these parameters to improve in 6 weeks because other studies of change in lung function are over a much longer period of time. Normal decline in lung function in control group of healthy subjects has been reported as a drop in FEV<sub>1</sub> of 35mls/year (Peat et al., 1987), in comparison to people with COPD who have a mean drop of 65mls/year (Pauwels et al., 1999). Statins appear to slow the rate of decline in lung function in current and ex-smokers independent of underlying lung disease, over a long period of time of ≥6 months; however, this evidence is retrospective (Keddissi et al., 2007).

The pleiotropic nature of statins, including being anti-inflammatory has been demonstrated in animal models. When rats were given simvastatin either prior to and during, or just during tobacco smoke exposure, those who had simvastatin prior to smoke exposure had a reduced number of inflammatory mediators including neutrophils in the lung and airways (Davis et al., 2012). The use of statins in smoke-induced lung disease has been shown, but the need for primary prevention is evident (Davis et al., 2012). This is reinforced by another clinical study showing patients with COPD who have recently been on statin therapy, or those

with higher average daily doses have been found to have a decreased risk of COPD exacerbation requiring hospitalization (Wang et al., 2013). As lowering the increased inflammation seen in patients with COPD is thought to be disease modifying, it is worth exploring the use of other therapeutic drugs that are known to lower circulating inflammation, and thus slow disease progression.

Although there was no improvement in functional tests it was encouraging that there was no decline in results in the active group compared to placebo, as musculoskeletal side effects are common in those on statin therapy (Bruckert et al., 2005).

#### **4.4.4      Limitations**

Airway inflammatory markers could not be performed in all subjects; therefore the lack of significance detected in this study could be due to the study ending up being underpowered. The study was underpowered to detect changes in inflammatory markers following 6 weeks intervention with a statin compared to a placebo. It is possible that different measures of circulating inflammation may detect a change that we did not with the markers we used. Six weeks is not long enough to detect a change in lung function.

#### **4.4.5      Conclusion**

In this RCT, there was not a detectable change in either the specific airway or circulating inflammatory markers we selected, in the active compared to placebo group following 6 weeks treatment with active compared to placebo. Airway inflammatory markers were only in a limited number of subjects. Neither were we

able to detect any change in the functional assessments we performed in the active compared to placebo group after 6 weeks intervention.



# **Chapter 5**

**Relationship of haemodynamics to  
airway and systemic inflammatory  
measures in patients with COPD**

## **5 Relationship of haemodynamics to airway and systemic inflammatory measures in patients with COPD**

### **5.1 Introduction**

The cardiovascular morbidity associated with COPD has led to a number of in-depth studies to determine potential associations and likely mechanisms. A non-invasive predictive measure of cardiovascular disease is aortic stiffness, which has been shown to be elevated in patients with COPD compared to controls (Sabit et al., 2007), in other chronic inflammatory conditions (Wang et al., 2005, Mäki-Petäjä et al., 2006), and in those with traditional CV risk factors (Laurent et al., 2001). Reasons behind the increased aortic stiffness in patients with COPD have been suggested and include systemic inflammation (Sabit et al., 2007), loss of elasticity (Maclay et al., 2009), aortic calcification (Bolton et al., 2011) and sympathetic drive (Volterrani et al., 1994, Andreas et al., 2005). Increased arterial stiffness has been reported to be influenced by age, blood pressure and thoracic aortic calcification (Bhatt et al., 2014).

There is evidence of increased systemic inflammation in COPD including CRP (Sin and Man, 2003) and IL-6 (Ferrari et al., 2013). Levels of inflammatory markers such as fibrinogen are increased in patients with COPD when compared to smoker and never smoker controls (Miller et al., 2013).

At times of exacerbation, there are further rises in cytokines from this increased baseline inflammatory state (Wedzicha et al., 2000, Bhowmik et al., 2000). The cause increased inflammation is not known, but hypotheses such as overspill from the lungs into systemic circulation, has been suggested (Gan et al., 2004, Fujii et al., 2002), but this is controversial (Sevenoaks and Stockley, 2006, Vernooij et al., 2002, Sapey et al., 2009). Whether or not systemic inflammation stems from airway spill-over, there is a spectrum of airway inflammation in COPD. This can be measured in a number of ways - with pertinent mediators in COPD being neutrophils and macrophages.

CV disease is common in patients with COPD and was associated with increased mortality and increased systemic inflammation (Soriano et al., 2005, Fabbri et al., 2008). Interestingly in 3,164 COPD patients, 337 smokers and 245 non-smoker controls there was no association with CRP and CV comorbidity as self-reported in the ECLIPSE study (Miller et al., 2013).

Increased vascular wall inflammation in patients with COPD has been reported indirectly using PET-CT, which may provide more mechanistic direction as to how systemic inflammation contributes to the increased CV risk seen in COPD (Coulson et al., 2010).

Results of whether specific isolated circulating inflammatory mediators relate to aortic PWV have been variable with Sabit *et al.* reporting an association with IL-6, whilst Maclay *et al.* found no association with CRP (Sabit et al., 2007, Maclay et al., 2009). McAllister *et al.* also did not find an association between arterial stiffness and CRP in patients with COPD, however they used brachial PWV to assess arterial

stiffness rather than aortic PWV (McAllister et al., 2007). That said, CRP is a more acceptable measure of inflammation in the CV literature with relationships of CRP to aortic stiffness shown in apparently healthy individuals (Yasmin et al., 2004) and a general population of older men in the Caerphilly study, however, it is not a proven causal relationship (Schumacher et al., 2009).

Circulating levels of MMP-9 are increased in patients with COPD compared to controls, with reports suggesting increased levels particularly in patients with osteoporosis (Bolton et al., 2009b). Maclay *et al.* tested the hypothesis that increased aortic stiffness in COPD might be due to systemic elastin degradation (Maclay et al., 2009). Circulating MMP-9 has been reported as being an enzyme that can degrade the elastic component of the arterial wall (Yasmin et al., 2005) and therefore contribute to aortic stiffness. Circulating levels of MMP-9 in COPD have not been determined in relation to aortic stiffness; but in other populations, MMP-9 has been related to aortic stiffness (Yasmin et al., 2005). A linear correlation between circulating MMP-9 and aortic PWV has been shown in patients with never treated hypertension (Zhou et al., 2007), isolated systolic hypertension and apparently healthy subjects (Yasmin et al., 2005). Conversely, Vlachopoulos *et al.* reported an inverse relationship between serum MMP-9 levels and aortic stiffness as measured by aortic PWV after adjusting for confounding variables, however, the population appear to be younger and more healthy as they have lower baseline aortic PWV and CRP levels (Vlachopoulos et al., 2007).

Endothelial NO is a vasoactive substance which influences vascular tone, and basal NO production has been shown in animal models to influence large artery

distensibility (Wilkinson et al., 2002b). Supportive findings of increased basal NO have been shown in a small double-blinded RCT in 8 healthy males with a mean age of 30 years where the vascular measure of basal NO synthesis modulated large artery stiffness, as measured by Aix, rather than the preferred method of PWV (Wilkinson et al., 2002a).

We have previously reported that patients with COPD and more purulent sputum as self-reported have increased aortic stiffness (John et al., 2012). That study was clinically self-reported based as opposed to having objective measures such as sputum cell counts. Aortic PWV has been related to infection and inflammation in patients with COPD at the time of exacerbation, where aortic PWV was strongly correlated with airway IL-6, from spontaneous sputum samples (Patel et al., 2012). A recent study in 259 participants looking at salivary biomarkers of inflammation found a positive correlation between salivary CRP and aortic PWV, and therefore an alternative means to assess CV risk (Labat et al., 2013), however, the study was performed in a general population and not patients with COPD. A multivariate analysis found an association with both salivary CRP and MMP-9 and carotid intima-media thickness (CIMT), but not with MMP-9 and PWV (Labat et al., 2013).

In this chapter we set out to determine whether there was a relationship between circulating and airway inflammatory mediators and aortic stiffness in a well-defined group of patients with COPD without co-existent hypercholesterolaemia or known diabetes mellitus or IHD, in a cross-sectional manner.

**Hypothesis:**

We hypothesised that the previously reported associations of circulating CRP and aortic PWV in a general population would be demonstrated here in clinically stable patients with COPD. Further, we wished to explore the nature of circulating to pertinent airway inflammatory markers and aortic stiffness.

## 5.2 Methods

Data for this chapter was baseline results n=70 from the study 'Cardiovascular and Inflammatory Effects of Statin Therapy in COPD', as previously described, see methods 2.1.

Test methodologies pertinent to this chapter are aortic stiffness as determined by aortic PWV (methods 2.2.2), sputum samples (methods 2.2.5), exhaled nitric oxide (methods 2.2.4) and venepuncture for circulating inflammatory markers hs-CRP and MMP-9 (methods 2.2.7.c). All subjects gave written informed consent prior to data collection.

Subject recruitment has been previously described, see methods 2.1.3. There were extensive inclusion and exclusion criteria to check eligibility into the study, see methods 2.1.1.

Data was analysed using Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 21.0, with  $p < 0.05$  considered significant. Non-normally distributed data was log transformed where possible to allow parametric testing to be performed. Pearson correlation coefficients were used to investigate the relationship, if any, and measure the strength of the linear relationship between two variables. The difference in mean value between 2 groups was analysed using a t-test, and ANOVA with post-hoc analysis was used to compare means in 3 or more groups.

Analysis was performed where subjects were grouped according to their aortic PWV values with a value of  $< 8\text{m/s}$  being low,  $8\text{-}10\text{m/s}$  normal and a value  $> 10\text{m/s}$

regarded as high. A sub-group analysis looking at subjects with an aortic PWV >10m/s was also performed.

Further associations were explored in a sub-group of subjects with a high hs-CRP, defined as >3mg/l.



## 5.3 Results

Baseline demographics, and airway and circulating inflammatory markers, for the total group in the statin RCT are shown in Table 5.1.

### 5.3.1 Circulating Inflammatory Markers

There were no associations between circulating markers of inflammation and aortic stiffness; specifically log hs-CRP and aortic PWV,  $r=-0.19$ ,  $p=0.12$ ,  $n=70$  and circulating MMP-9 and aortic PWV,  $r=-0.04$ ,  $p=0.74$ ,  $n=69$ .

When subjects with a high hs-CRP values ( $>3\text{mg/l}$ ) were looked at there was no correlation with aortic PWV,  $r=-0.13$ ,  $p=0.47$ ,  $n=36$ .

Aortic PWV was compared between those with hs-CRP  $\leq 3\text{mg/l}$  and  $>3\text{mg/l}$ . There was no significant difference in aortic PWV where those with a hs-CRP  $\leq 3\text{mg/l}$  had an aortic PWV of  $10.1 (3.0)\text{m/s}$  and those with a hs-CRP  $>3\text{mg/l}$  had an aortic PWV of  $9.0 (2.8)\text{m/s}$ ,  $p=0.12$ .

When hs-CRP was compared across the 3 groups of aortic PWV ( $<8\text{m/s}$  ( $n=21$ ),  $8-10\text{m/s}$  ( $n=24$ ) and  $>10\text{m/s}$  ( $n=25$ )) there were no significant differences in mean hs-CRP across any of the aortic PWV groups,  $p=0.25$ ; however, there was a trend suggesting that with lower aortic PWV, surprisingly hs-CRP was higher, see Figure 5.1.

When circulating MMP-9 was compared across the 3 groups of aortic PWV ( $<8\text{m/s}$ ,  $8-10\text{m/s}$  and  $>10\text{m/s}$ ) there were no significant differences in mean MMP-9 across any of the aortic PWV groups,  $p=0.19$ ; neither did there appear to be any trend. See Figure 5.1.

When the group is divided into high and low MMP-9 levels based on median MMP-9 result of 52.4ng/ml, there was no significant difference in aortic PWV between those with a MMP-9 value below the median MMP-9 measurement compared to those with a value above the median value, 9.9 (2.8)m/s and 9.2 (3.1)m/s respectively,  $p=0.34$   $n=69$ .

**Table 5.1 Baseline Demographics, Airway and Circulating Inflammatory Markers in the Total Group**

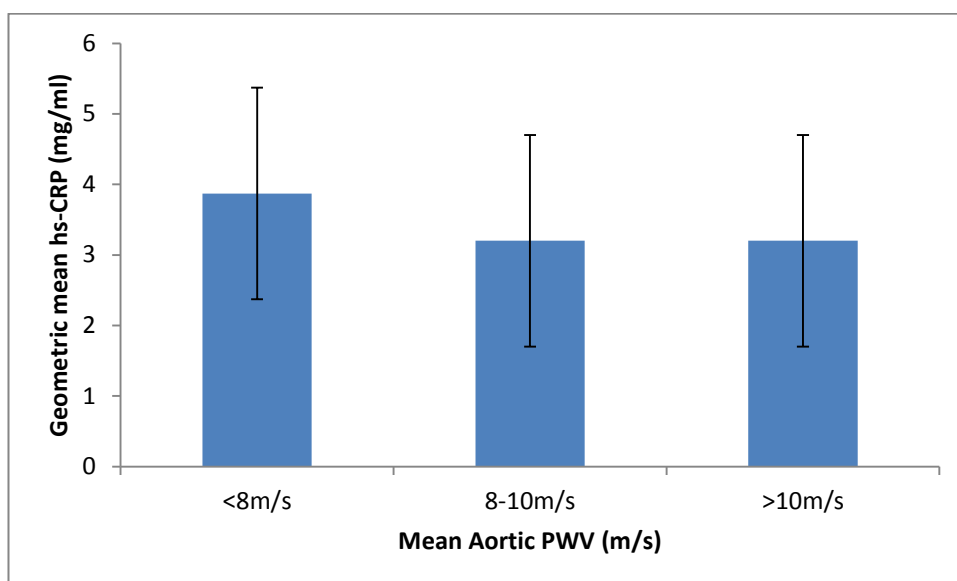
Demographics / Inflammatory marker		Mean (SD)
Age (years)		64 (8)
Gender Male n(%)		50 (71)
FEV <sub>1</sub> (l)		1.5 (0.6)
FEV <sub>1</sub> % predicted		54 (14)
Smoking pack years		47 (23)
Aortic PWV (m/s)		9.6 (2.9)
Peripheral systolic BP (mmHg)		148 (23)
Peripheral diastolic BP (mmHg)		88 (12)
Alx (%)		27 (9)
Total cholesterol (mmol/L)		5.3 (0.8)
Circulating hs-CRP (mg/l) n=70 †		3.2 (2.9)
Circulating MMP-9 (ng/l) n=69		56.9 (17.1)
Sputum neutrophils n=27	Actual cell count (number)	323 (70)
	Percentage	80 (18)
Sputum macrophages n=27 †	Actual cell count (number)	35.5 (3.2)
	Percentage	10.2 (2.9)
FeNO50 (ppb) † n=61		15.7 (2.1)
FeNO Alveolar gradient n=57		8.6 (9.5)
FeNO Bronchial intercept n=57		35.6 (28.9)

† geometric mean

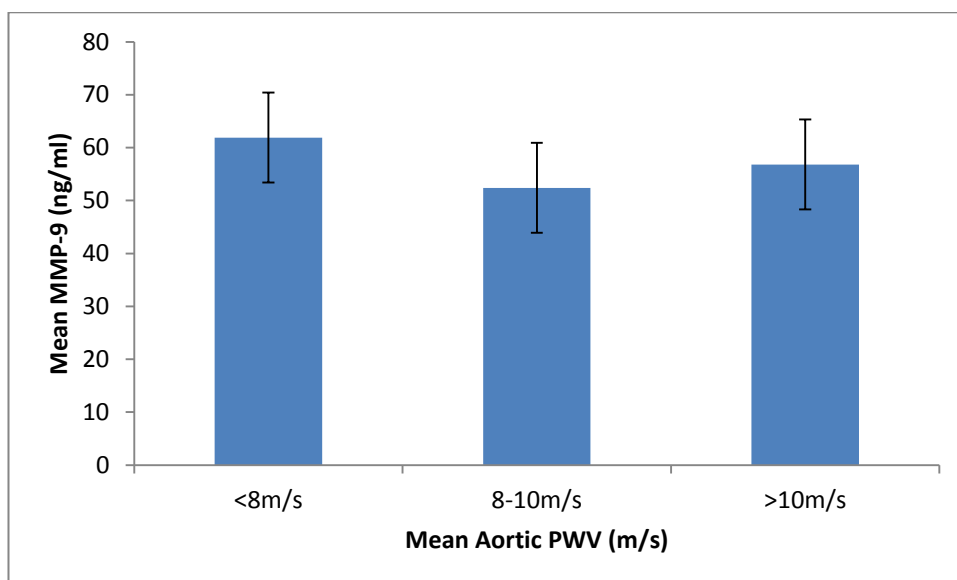
**Abbreviations:** **FEV<sub>1</sub>**: forced expired volume in 1 second, **PWV**: pulse wave velocity, **BP**: blood pressure, **Alx**: augmentation index, **hs-CRP**: high sensitivity C-reactive protein, **MMP-9**: matrix metalloproteinase-9, **FeNO**: fraction of exhaled nitric oxide

**Figure 5.1 Baseline Circulating Inflammation for Total Population Grouped by Baseline Aortic PWV**

a) hs-CRP



b) MMP-9



**Abbreviations:** **hs-CRP:** high sensitivity C-reactive protein, **MMP-9:** matrix metalloproteinase-9, **PWV:** pulse wave velocity

Bar – mean value

Error bars – Standard deviation

### **5.3.2      Airway Inflammatory Markers**

There was no correlation in sputum neutrophil or macrophage percentage and aortic PWV,  $r=-0.23$   $p=0.24$ , and  $r=0.21$ ,  $p=0.30$ ,  $n=27$  respectively. Neither was there any correlation between actual sputum neutrophil or macrophage cell counts and aortic PWV,  $r=-0.19$ ,  $p=0.36$ , and  $r=0.22$ ,  $p=0.27$ ,  $n=27$  respectively.

There was no correlation between aortic PWV and any of the exhaled nitric oxide measurements – FeNO50, alveolar gradient and bronchial intercept,  $r=-0.002$ ,  $p=0.99$ ,  $n=61$ ,  $r=-0.01$ ,  $p=0.97$ ,  $n=57$  and  $r=-0.14$ ,  $p=0.31$ ,  $n=57$  respectively.

Tertile analysis, where the population was divided into thirds and therefore a high, middle and low result group, based on FeNO50 measurements, showed no difference between different tertiles and aortic PWV,  $p=0.67$ .

### **5.3.3      Aortic PWV >10m/s Sub-group Analysis**

In those with a baseline aortic PWV >10m/s ( $n=25$ ) there was no correlation with aortic PWV and circulating hs-CRP  $r=0.04$ ,  $p=0.85$ , or between aortic PWV and circulating MMP-9,  $r=0.20$ ,  $p=0.33$ . Further, there were no associations of aortic PWV with sputum neutrophils, whether actual cell counts or percentages,  $r=-0.40$ ,  $p=0.25$ ,  $n=10$  and  $r=-0.40$ ,  $p=0.26$ ,  $n=10$  respectively; or between aortic PWV and sputum macrophages whether actual cell counts or percentages,  $r=0.33$ ,  $p=0.36$ ,  $n=10$  and  $r=0.36$ ,  $p=0.31$ ,  $n=10$  respectively. There were no significant correlations of aortic PWV and any of the exhaled nitric oxide parameters-FeNO50  $r=0.24$ ,  $p=0.29$ ,  $n=22$ , alveolar gradient  $r=0.16$ ,  $p=0.50$ ,  $n=20$  and bronchial intercept  $r=0.04$ ,  $p=0.87$ ,  $n=20$ .

## 5.4 Discussion

In this well-defined group of stable patients with COPD, without CV disease or diabetes, we could not find any associations between aortic stiffness and either airway or circulating inflammatory mediators. All patients were at clinical stability which is important as inflammatory markers (Bhowmik et al., 2000) and aortic PWV (Patel et al., 2013) have been shown to increase during exacerbations.

Airway inflammation was assessed using exhaled nitric oxide measurements and sputum samples whilst circulating inflammation was assessed using serum hs-CRP and MMP-9. We were unable to detect any significant difference in aortic PWV between those with a high and low hs-CRP where 3mg/l was used as the cut-off (Lahousse et al., 2013); similarly we were unable to detect any difference in hs-CRP when subjects were grouped according to baseline aortic PWV.

It was surprising not to find any associations between hs-CRP and aortic PWV. Both CRP (Sin and Man, 2003) and aortic PWV (Sabit et al., 2007) are well described markers of cardiovascular risk in patients with COPD. CRP is a preferred inflammatory marker of CV risk and strong, independent associations have been shown between CRP and CV disease in the general male population and in a general apparently healthy cohort (Ridker et al., 1997, Ballantyne et al., 2004). Associations have also been shown between CRP and aortic PWV as a marker of arterial stiffness in the general population (Schumacher et al., 2009, Kullo et al., 2005, Mattace-Raso et al., 2004). In a study of 790 healthy males CRP was shown to be a predictive marker of CV risk, but a causal role in the development of arterial stiffness as measured by aortic PWV could not be established (Schumacher et al., 2009).

Conversely, studies have also reported no association between CRP and aortic PWV in a large general population study of 1078 subjects (Nishida et al., 2007), a cross-sectional study of 157 patients with COPD, however, they used brachial PWV rather than aortic PWV which is less predictive of CV risk (McAllister et al., 2007), and in a smaller study of 18 males with COPD (MacLay et al., 2009), in keeping with our findings.

The inflammatory markers hs-CRP and MMP-9 measured in this group of 70 patients with COPD shows an increased inflammatory state. We chose to divide the population based on a CRP value of 3mg/l because there have been lots of published studies using this cut-off point, and a value above 3mg/l has been shown to relate to CV risk (Lahousse et al., 2013), and although a range, our geometric mean hs-CRP was 3.2mg/l, which demonstrates increased circulating inflammatory markers in COPD. This was not a well-defined consensus because there were varying cut-off points used and suggested in other publications (Ridker et al., 2005a). Hs-CRP was regarded to be more sensitive to determine levels of inflammation within the normal range than standard CRP (Ridker, 2001).

We grouped our subjects using their baseline aortic PWV measurements, based on previously reported suggestions for low (<8m/s), normal (8-10m/s) and increased (>10m/s) readings; but as 'normal' values for aortic PWV are not well defined, and higher values such as aortic PWV >12m/s have been suggested for increased aortic stiffness (Phababpha et al., 2013, Mancia et al., 2007), the cut-offs we have chosen may impact on our findings. When hs-CRP was compared between the 3 groups of aortic PWV there appeared to be a correlation, with a surprising trend towards

higher hs-CRP in those in the lowest aortic PWV group, however, this did not reach significance.

MMP-9 has been shown to relate to aortic PWV in healthy individuals (Yasmin et al., 2006, Yasmin et al., 2005), and in patients with diabetic chronic kidney disease (CKD) (Chung et al., 2009) but we could not find the same relationships in our COPD group. Chung *et al.* reported baseline arterial stiffness in diabetes and CKD of 11.9 +/- 4.6m/s, which was much higher than the PWV we had in our study group of patients with COPD, and 75% of the diabetic CKD patients were taking a statin, however, this is a different disease population to our reported patient group (Chung et al., 2009).

There is a wealth of evidence showing endothelial NO affects sympathetic tone, and this modulation of vascular compliance has been shown in animal models to be independent of BP changes (Fitch et al., 2001), however, exhaled measurements have not been explored and it is therefore a leap of faith relating exhaled NO to measures of aortic stiffness. Aortic stiffness is dependent on smooth muscle tone, of which the functional regulation of arterial stiffness is reliant on both local and circulating vasoactive substances, with NO synthesis shown in vivo to modulate large artery stiffness (Wilkinson et al., 2002a).

Sputum neutrophilia is well defined (Spanevello et al., 2000), and a common presentation in patients with COPD which was also demonstrated in our population. Patients with COPD self-reported increased sputum production has been associated with increased aortic PWV (John et al., 2012). When we looked at objective measures such as neutrophil cell counts in sputum samples we did not



find the same association. This could be due to our sputum samples being collected at clinical stability, whereas the previously reported subjective measures would have been recalled over a longer duration rather than a single sample; and the objective measure was neutrophil counts which does not equate to increased sputum production and therefore another measure such as sputum volume may be more representative. It has been recently reported that in patients with COPD with evidence of airway bacteria or rhinovirus, there was no increase in aortic PWV when compared to those at clinical stability; however during infective exacerbations of COPD there was a greater rise in aortic PWV from stable clinical state compared to those without infection or sputum production (Patel et al., 2013).

There are several points to consider as to why we didn't find any associations between our airway and circulating inflammatory markers and aortic PWV. Our aortic PWV results were lower than we were expecting. Results published by Schumacher *et al.* where they found an association between aortic PWV and CRP had a higher aortic PWV of 11.5, in a study of 800 men in Caerphilly (2.8)m/s (Schumacher et al., 2009), however, despite being in a general population, of key importance they did not exclude those with previous CV disease; therefore our different population may be a reason for a lack of correlation. The nature of our study means that the study population was a very select group of patients with COPD, therefore the results may not be representative of the general COPD population. Importantly subjects with CV disease were excluded from the study; therefore the increased inflammation shown was a reflection of the increased

inflammatory state seen in patients with COPD and not a result of previous CV history.

A limitation to this study is that a power calculation was not performed based on any cross-sectional analysis, as this data was baseline data from an RCT, therefore the power calculation was performed based on detecting a change after intervention. It is not known if the analysis is adequately powered, especially given FeNO and sputum induction was only performed in a subset of the main group. Sputum samples were only available in a subset of patients due to the protocol for sputum induction and some patients being unable to produce an adequate sample, hence the sample size may impact upon our findings.

Patients were excluded from the study if they were on oral steroids, however we could not control for inhaled steroids. The inhaled steroids may have dampened some of the inflammatory response as it was previously reported that 2 months treatment with beclomethasone dipropionate 500µg 3 times daily in patients with stable COPD significantly reduced sputum neutrophil cell counts (Confalonieri et al., 1998), and ICS have important immunomodulatory effects in patients with COPD, again reducing neutrophilic inflammation (Jen et al., 2012). Conversely, a study in patients with COPD found that following 4 weeks treatment with fluticasone propionate 500 µg twice daily there were no significant changes in inflammatory cell counts or neutrophil percentages, and MMP-9 results did not change, when compared to the placebo group (Culpitt et al., 1999).

In conclusion, we were unable to demonstrate any associations between the specific airway or systemic inflammatory markers and aortic stiffness in a group of well-defined patients with COPD, without hypercholesterolaemia, IHD or diabetes.

# **Chapter 6**

**Advanced Glycation End-products**

**– a Marker of Cardiovascular**

**Status in COPD?**

## **6 Advanced Glycation End-products – a Marker of Cardiovascular Status in COPD?**

### **6.1 Introduction**

At the moment, we are aware of the increased risk of cardiovascular (CV) disease and mortality in COPD but we do not assess the current CV state, predict the CV risk or consider preventative CV strategies. In order to address this increased CV risk in COPD patients a multi-dimensional approach is needed. A shift in patient care is required so that rather than just assessing lung function, other measures are made to address CV risk (Jones and Agusti, 2006).

A potential new biomarker for the detection of those patients with COPD at increased CV risk is Advanced Glycation End-products (AGE), which are predictive of CV risk in other disease groups (Meerwaldt et al., 2004), and as they contribute to structural vascular wall changes may be a link between COPD and CV disease (Basta, 2008).

AGEs are markers of glycaemic and oxidative stress, pro-inflammatory and alter structure through collagen cross-linking, formed through the Maillard reaction - a chemical reaction between an amino acid and a reducing sugar. Proteins such as collagen and elastin are particularly prone to AGE modification (Wu et al., 2011). AGEs accumulate with chronological age under normal conditions and have been found to contribute to the pathogenesis of multiple age related diseases, for example – established CV disease, diabetes and renal failure. Semba *et al.*

identified the specific AGE – plasma carboxymethyl-lysine (CML) as a predictor of all-cause CV disease mortality, in a population based study looking at adults over the age of 65 years (Semba et al., 2009a). AGE receptors (RAGE) belong to the immunoglobulin super-family and can be found on many cell types, including endothelial cells and macrophages. RAGE can be either membrane bound or soluble, with membrane-associated receptors accounting for approximately 80% of lung RAGE (Hudson et al., 2008).

There has been recent interest in AGE and its receptor: RAGE in both the airways (Ferhani et al., 2010) and circulating (Smith et al., 2011) in subjects with COPD. Smokers reportedly have increased levels of the membrane bound receptor RAGE in their proximal and distal airways (Ferhani et al., 2010). Whereas a recent study by Sukkar *et al.* reported that a lower systemic soluble RAGE (sRAGE) was associated with neutrophilic asthma and COPD (Sukkar et al., 2012). These subjects with neutrophilic COPD or evidence of airway bacterial colonisation also had a reported lower mean systemic sRAGE in comparison to those without, suggesting sRAGE levels vary with COPD phenotype (Sukkar et al., 2012). As RAGE contributes to tissue inflammation and remodelling, it may impact upon the pathogenesis and progression of COPD (Ferhani et al., 2010). A recent GWAS by Repapi *et al.* found that in a population of 20,288 – including two historical Nottingham based populations, over and above the impact of smoking, the single nucleotide (SNP) rs2060700 encoding RAGE polymorphism is associated with lung function, especially the ratio of FEV<sub>1</sub>/FVC (Repapi et al., 2010).

In studies looking at AGE levels in COPD the interaction between RAGE and AGE is thought to contribute to the pathogenesis (Wu et al., 2011). A case control study by Miniati *et al.* found statistically significant lower sRAGE levels in COPD subjects compared to an age and sex matched control group. A significant inverse relationship was also found between sRAGE and the degree of airflow obstruction as defined by FEV<sub>1</sub> in patients with COPD (Miniati et al., 2011). These results are supportive of the findings previously reported by Smith *et al.* where it was reported that sRAGE was decreased in COPD compared to controls, and decreased further during acute exacerbations of COPD, and the same negative association with airflow limitation (Smith et al., 2011). An association between sRAGE and transfer factor of carbon monoxide (TLco) and degree of emphysema as defined using a Computed Tomography (CT) scan was also reported (Miniati et al., 2011). sRAGE is an anti-inflammatory molecule and as such reduced levels of sRAGE have been reported in other chronic inflammatory diseases such as rheumatoid arthritis (Pullerits et al., 2005) and coronary atherosclerosis (Falcone et al., 2005).

Skin autofluorescence (AF) permits a non-invasive measurement of skin AGE, validated against the gold standard method of skin biopsies (Meerwaldt et al., 2004). These measurements are made using an AGE reader, which measures the accumulation of AGE in tissue, providing a quick, non-invasive measure, however specific individual AGE's cannot be measured. Skin levels reflect accumulation of oxidative stress within the tissue, unlike circulating levels which are more variable and are affected by smoking (Cerami et al., 1997) and diet (Uribarri et al., 2007). If

a relationship was found, measurements of skin AGE could be easily integrated into the clinical setting to assess cardiovascular risk.

Skin AGE levels have been reported to be significantly increased in type 2 diabetes (Lutgers et al., 2006) and renal failure (Hartog et al., 2005). Previous research has found relationships between AGE and other cardiovascular risk measures such as arterial stiffness (Ueno et al., 2008). In a chronic kidney disease population skin AGE results have been shown to be independently associated with CV and renal risk factors (McIntyre et al., 2011). A study conducted in Nottingham by McIntyre *et al.* found that in 115 established dialysis patients metabolic stress and hyperglycaemia resulted in elevated skin AGE levels compared to an age-matched non-chronic kidney disease database (McIntyre et al., 2009). Skin autofluorescence (AF) has been reported to be a useful clinical adjunct when evaluating both fatal and non-fatal CV events, and total mortality in type 2 diabetics (Lutgers et al., 2009). In 881 stable type 2 diabetics in primary care, it has been reported that skin AGE levels, measured by the AGE reader, are predictive of those who developed micro-vascular complications, neuropathy and micro-albuminuria; with increased AGE values having a positive correlation with increased risk of complications (Gerrits et al., 2008).

More recently, in The Netherlands, with a population of 88 patients with COPD and 55 controls, it was reported that skin AGE and some specific circulating AGE's are higher in patients with COPD compared to controls, and inversely associated with disease progression (Gopal et al., 2014).



**Hypothesis:**

**Skin AGE is elevated in patients with COPD compared to controls with a smoking history, independent of smoking and age. Skin AGE is inversely associated with spirometric measures of lung function and directly associated with aortic stiffness.**

## 6.2 Methods

Personnel: MJ co-ordinated the study and was responsible for the data entry, clinical conduct of the study and day-to-day management of the project. MJ led on the preparation for NRES and R&D submissions. All procedures were performed by an appropriately trained member of staff, either MJ or SH, according to local standard operating procedures (SOPs). Enzyme-Linked Immunosorbent Assay (ELISA) testing for serum AGE was performed by SS, Department of Immunology, University of Nottingham, and for RAGE was performed by WC (see acknowledgments).

### 6.2.1 Trial Design

This study is a cross-sectional case control pilot study. The objective of the study was to quantify skin AGE as a potential biomarker.

Inclusion criteria:

- Male or female
- Aged between 40-85 years
- Caucasian (skin AGE assessment only valid in Caucasian skin)
- Greater than 10 pack years smoking history
- Formal diagnosis of COPD (patient group) or no respiratory disease (controls)

Exclusion criteria:

- Known alpha 1 antitrypsin deficiency
- Active or suspected malignancy
- Terminal disease not likely to survive 6 weeks
- Any other lung conditions other than COPD where relevant

There was also a never smoker control group. The inclusion and exclusion criteria were the same with the exception of the smoking criteria.

### **6.2.2      Statistics**

All data was analysed using Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 21.0. GraphPad Prism 5 (Graphpad software. San Diego, CA) was used for some figures. Associations between categorical data were analysed using chi-squared ( $\chi^2$ ) test. Normally distributed continuous variables were analysed using an independent t-test. One-way analysis of variance (ANOVA) tests were performed if there were more than two sample sets. Unless otherwise specified, arithmetic mean and standard deviation was used to show the measure of central tendency. Where possible, non-parametric data – serum AGE and serum soluble RAGE (sRAGE), was  $\log_{10}$  transformed and parametric testing performed. Pearson correlation coefficients were used to measure the strength of linear relationships between two variables. Stepwise multivariable linear regression was used to estimate the effect of COPD on other variables such as skin AGE and presence of comorbidities. A  $p < 0.05$  value is considered statistically significant. Bland-Altman plots were used in the analysis of agreement in the validation of vicorder operators. All data was first analysed looking at the COPD and control

group together. In some cases, further sub-group analyses were performed looking at the COPD group alone.

#### *6.2.2.a Power calculation*

A formal power calculation could not be performed as there was no previous pilot data of skin AGE in patients with COPD with which to calculate a sample size. Pragmatically, we proposed to recruit 60 patients with COPD, 30 smoker controls and 30 never smokers; matched for age and gender for this pilot study.

### **6.2.3      Approvals**

#### *6.2.3.a Ethics*

Research Ethics Committee (REC) approval was granted on 18<sup>th</sup> November 2010. All relevant documentation was reviewed by the REC including participant information sheets – Appendix 4, letter of invitation to participant, protocol, consent forms, poster advertisements and questionnaires.

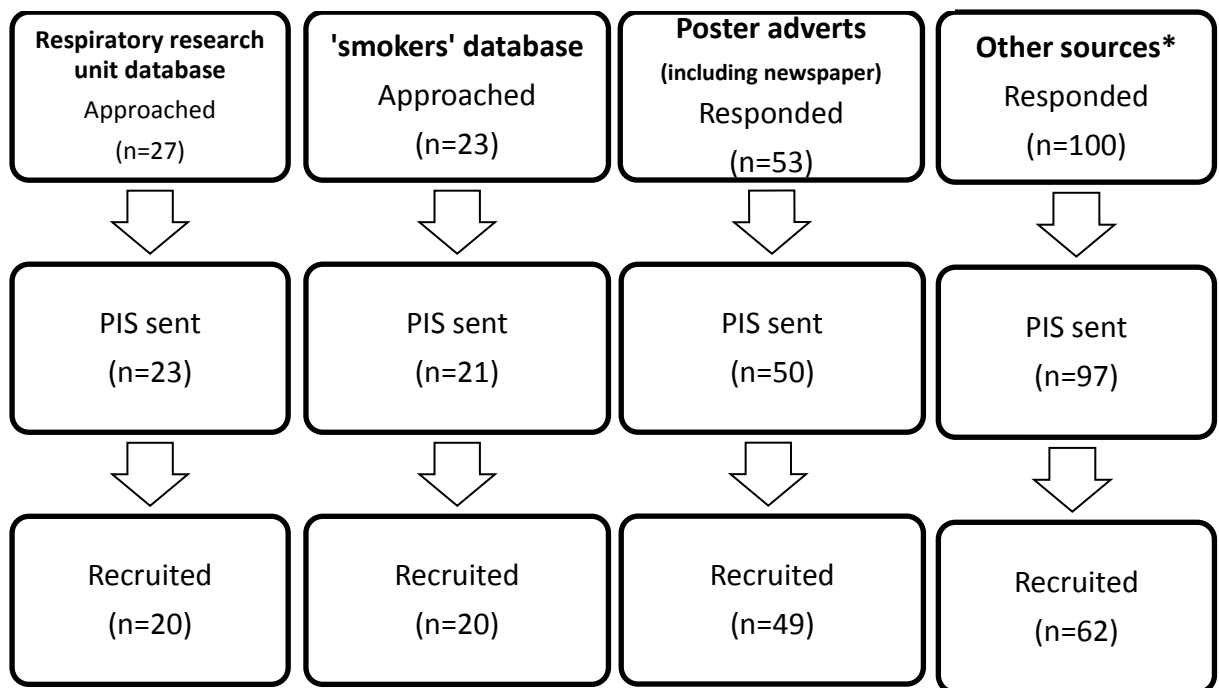
#### *6.2.3.b Research and Development (R&D)*

R&D was granted on 26<sup>th</sup> November 2010. All relevant documentation was reviewed including protocol, consent forms, participant information sheets, advertisements, questionnaires and GP letters.

### **6.2.4      Recruitment**

Subjects were recruited from out-patient clinics, poster advertisements across the Nottingham University Hospital NHS Trust, University of Nottingham and the local newspaper, Nottingham Respiratory Research website, pulmonary rehabilitation programmes, the respiratory research unit database and the 'Nottingham smoker' database (see Figure 6.1). All subjects were assessed at clinical stability.

**Figure 6.1 Recruitment Source for the 151 who Consented**



\*Other sources include pulmonary rehabilitation sessions, consultant clinics, other departmental studies, friends/relatives, departmental website and Breathe Easy meetings.

**Abbreviations-** PIS: patient information sheet

### **6.2.5      Study Outline**

Informed, written consent was sought from all participants. All procedures were performed during a single study visit in the Clinical Trials Unit, City Hospital, NUH NHS Trust. Where possible, participants were asked to refrain from using short acting  $\beta_2$  agonist bronchodilators for 4 hours prior to their study visit and to attend fasted for 6 hours.

### **6.2.6      Procedures**

#### *6.2.6.a    Medical history*

The participants were asked their medication, smoking history, medical conditions, physical activity, and occupational history.

#### *6.2.6.b    Haemodynamic measurements*

Peripheral blood pressure was measured in duplicate using an Omron automatic sphygmomanometer in the seated and supine position. All supine measurements were made after 10 minutes rest, see methods 2.2.2.a.

PWA was measured in duplicate to determine Alx using the Vicorder (SmartMedical, UK). When performing PWA the vicorder cuff was placed and inflated around the brachial pulse, see methods 2.2.2.b.ii.

Pulse wave velocity (PWV) was measured in duplicate using the vicorder. Both carotid-femoral (aortic) and brachial-femoral measurements were made; see methods 2.2.2.b.ii and Appendix 6.

#### *6.2.6.c Spirometry*

Spirometry was performed on a micro-medical spirometer (MicroLab MK6) to assess lung function. Measurements of Forced Expired Volume in 1 second (FEV<sub>1</sub>), Forced Vital Capacity (FVC) and FEV<sub>1</sub>/FVC ratio were recorded. Where evidence of airways obstruction was shown on baseline spirometry, reversibility was performed and post-bronchodilator spirometry recorded; see methods 2.2.3. The GOLD COPD classification was used (Decramer et al., 2013).

#### *6.2.6.d Skin AGE*

Skin AGE was measured in triplicate using an AGE Reader (DiagnOptics, Groningen) on the forearm of subjects. The average of the 3 recordings was taken as the final result. See methods 2.2.8 and appendix 7.

#### *6.2.6.e Blood samples*

Venepuncture was performed. Patients were asked to fast for 6 hours where possible. Samples were sent to pathology for renal function, glucose and a full lipid profile. Further blood was centrifuged, aliquoted and then stored in a -80°C freezer for later analysis of circulating AGE (Cell biolabs) and soluble RAGE (R&D systems, UK) levels as measured by ELISA see methods 2.2.7.

#### *6.2.6.f Questionnaires*

The MRC dyspnoea score, CAT score and SGRQ were completed by all subjects; see methods 2.2.15 and appendix 8-10.

#### *6.2.6.g Body composition*

Height (seca dual station, Hamburg, Germany) was measured. Weight and body composition (Tanita, Illinois, USA) including FM, FFM and total weight. BMI was calculated; see methods 2.2.13.

#### *6.2.6.h Carbon monoxide*

Exhaled CO levels were measured to assess smoking status using a Clement Clarke Smokerlyzer CO monitor (Harlow, UK); see methods 2.2.12.

#### *6.2.6.i Oxygen saturations ( $SpO_2$ )*

Oxygen saturation was measured using pulse oximetry with a Minolta Pulsox P300-P Pulse Oximeter (Tokyo, Japan); see methods 2.2.11.

### **6.2.7 Inter Operator Validation of Aortic PWV**

A validation study was performed to determine any inter-operator variability when performing measurements of aortic stiffness on the Vicorder. Both researchers performed arterial stiffness measurements on 10 randomly assigned subjects.

### **6.2.8 Validation of Skin AGE**

Skin AGE measurements were performed on 20 participants on three separate occasions to assess validity. Measurements were made in triplicate each time. Two sets of measurements were made consecutively on day 1 10 minutes apart, and a further set of results were recorded where possible at least 24 hours after the first set of measurements.



## 6.3 Results

In total, 202 people were approached regarding the study, 190 expressed an interest and had a PIS sent to them, 180 responded, and 151 consented and were entered into the study, see Figure 6.1. Of these, there were COPD  $n=84$  and smoker controls  $n=39$ . The main data analysis is between COPD subjects and an age and gender matched smoker control group. The 'control' group refers to both current and ex-smoker participants without any lung conditions. A small number of never smokers were also recruited for comparison but not included in the main analysis ( $n=20$ ). Of the 151, a small number ( $n=8$ ) were found to have other respiratory conditions often detected following spirometry and were excluded from the analysis. Of the subjects recruited, skin AGE measurements were not possible in 3 subjects - this was because the subjects had a dark sun tan and the machine was not able to perform the measurements - all of whom were in the control group. These 3 without skin AGE measurements were excluded from data analysis. The number entering the study was COPD  $n=84$  and controls  $n=36$ .

To note, aortic pulse wave velocity (PWV) was a latter addition following an amendment to the initial ethical approval so measurements were performed in 52 of the 84 COPD subjects and 35 of the 36 controls.

Although they were not excluded, there were 15 subjects with self-reported IHD (12 in the COPD group) and 13 with self-reported diabetes (9 in the COPD group).

The patients with COPD and the control group were matched for age, BMI and gender, see Table 6.1. Similar proportions were current smokers, but the COPD group had a great smoking pack year history.

In the COPD group the majority of subjects were GOLD stage 2. Resting oxygen saturations and carbon monoxide levels were similar across the 2 groups; see Table 6.1. The control never smokers are also shown here, though they were not used for the main analyses and were significantly younger than the COPD and control group.

**Table 6.1 Demographic Information of Subjects Recruited**

Mean (SD) (t-test unless otherwise stated)	Controls n=36	COPD n=84	P values	Never smoker n=20
Age (years)	65 (12)	68 (9)	0.19	58 (8)
Male / female (n) *	19/17	51/33	-	9/11
Smoking status (n) (never/ex/current) *	0/26/10	0/63/21	-	20/0/0
Smoking pack years	28 (17)	46 (27)	<0.001	0 (0)
FEV <sub>1</sub> (L)	2.58 (0.7)	1.5 (0.6)	<0.001	2.97 (0.7)
FEV <sub>1</sub> % predicted	98 (14)	58 (18)	<0.001	103 (13)
GOLD (1/2/3/4) (n)	-	6/54/20/4	-	-
Oxygen saturations (%)	96 (1)	95 (2)	<0.001	97 (1)
CO(ppm)	8 (10)	7 (9)	0.62	2 (2)
BMI (kg/m <sup>2</sup> )	27.3 (4.0)	27.5 (5.4)	0.89	25.4 (3.6)
FM (kg)	23.4 (7.3)	24.4 (10.1)	0.63	20.5 (8.0)
FMI (kg/m <sup>2</sup> )	8.3 (2.4)	8.5 (3.8)	0.72	7.3 (3.1)
FFM (kg)	54.5 (12.3)	54.0 (11.1)	0.81	52.2 (8.4)
FFMI (kg/m <sup>2</sup> )	19.0 (2.6)	18.6 (2.6)	0.56	18.1 (2.0)
Ischaemic Heart Disease n (%)	3 (8)	12 (14)	0.26	0 (0)
Diabetes Mellitus n (%)	4 (10)	9 (11)	0.94	0 (0)

Key: \* chi-squared test

Abbreviations: **BMI** body mass index, **FEV<sub>1</sub>** forced expired volume in 1 second, **CO** carbon monoxide, **FM** fat mass, **FMI** fat mass index, **FFM** fat free mass, **FFMI** fat free mass index, **GOLD**: global initiative for obstructive lung disease.

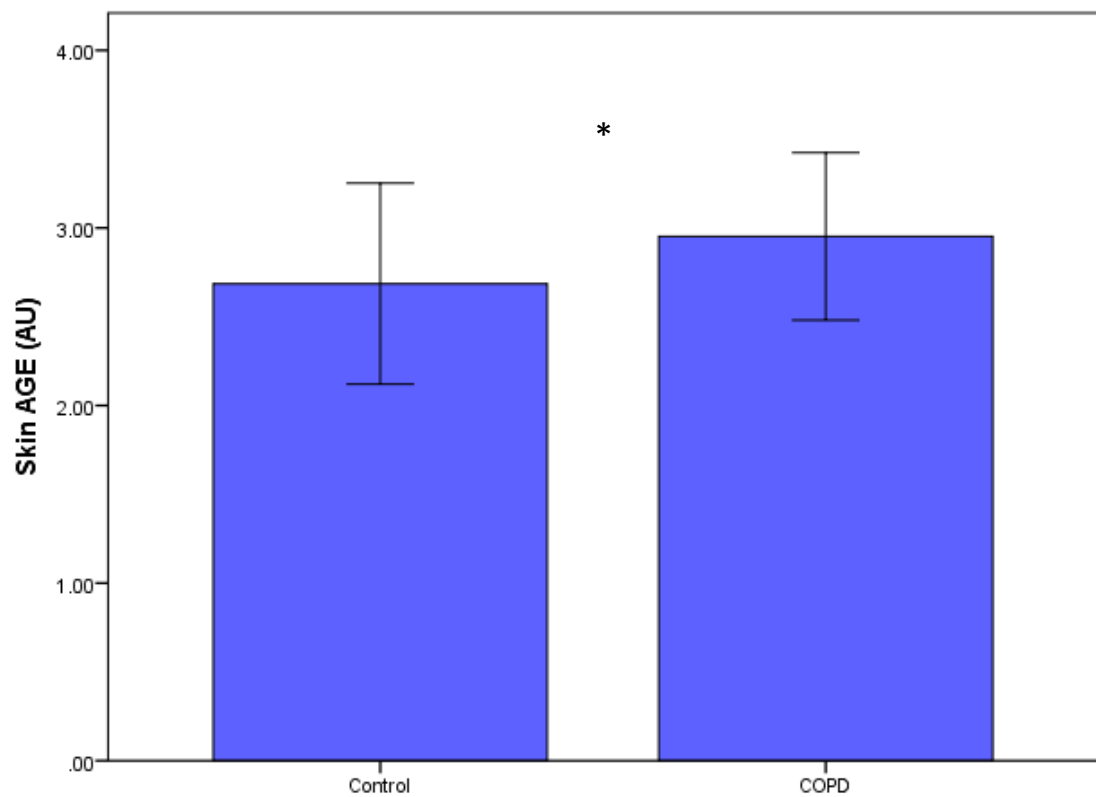
### **6.3.1      Skin AGE in COPD and According to Lung Function**

Skin AGE was greater in subjects with COPD, 3.0 (0.5) AU, compared to the control group, 2.7 (0.6) AU,  $p=0.04$ ; see Figure 6.2. The mean skin AGE for each of the GOLD stages 1-4 ( $n=86$ ) and the control group ( $n=36$ ) is shown in Figure 6.3. Although the mean skin AGE of smoker controls is lower than each of the GOLD stages, and there is a progressive increase in mean skin AGE levels as the GOLD stage increases, there was no statistical difference between any of the GOLD categories. There was only a small number of subjects recruited at GOLD stage 1,  $n=6$ , and GOLD stage 4,  $n=4$ ; compared to GOLD stage 2,  $n=54$ , and stage 3  $n=20$ .

There was an inverse relationship between FEV<sub>1</sub>% predicted and skin AGE  $r=-0.24$ ,  $p<0.01$  across the COPD and smoker control group, as a whole; see Figure 6.4. In the COPD group alone there was no significant correlation between FEV<sub>1</sub>% predicted and skin AGE.

Data analysis was performed to look at the difference in skin AGE results between current and ex-smokers across the COPD and smoker control group. There was no statistical difference in skin AGE results between current or ex-smokers with COPD, or between current and ex-smokers in the smoker control group. A positive correlation was found between skin AGE levels and smoking pack years, ( $r=0.22$ ,  $p<0.05$ ) across the whole group.

**Figure 6.2 Skin AGE in Controls and Patients with COPD**

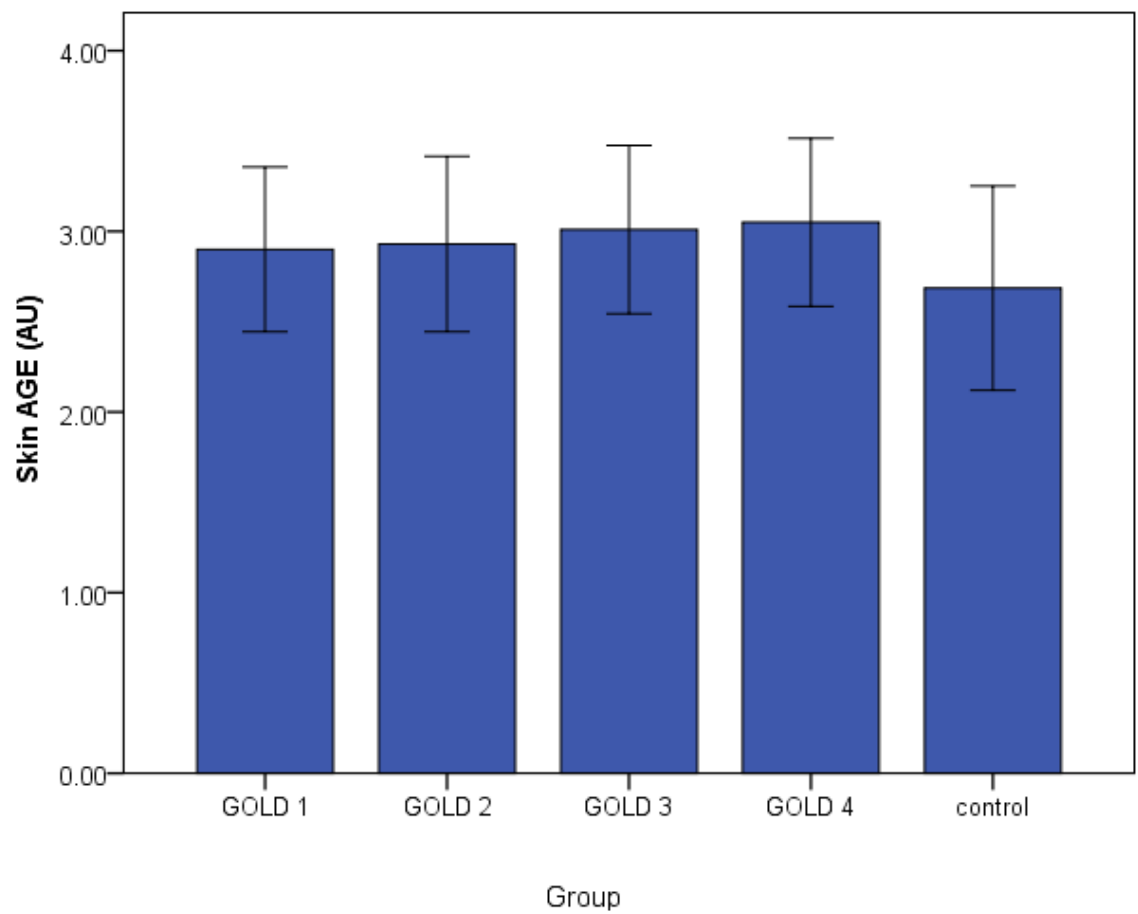


**Abbreviations – AGE:** advanced glycation end-products

**Key - \*:**  $p < 0.05$

The bars show the mean skin AGE for the control group and patients with COPD;  
the error bars show 1SD.

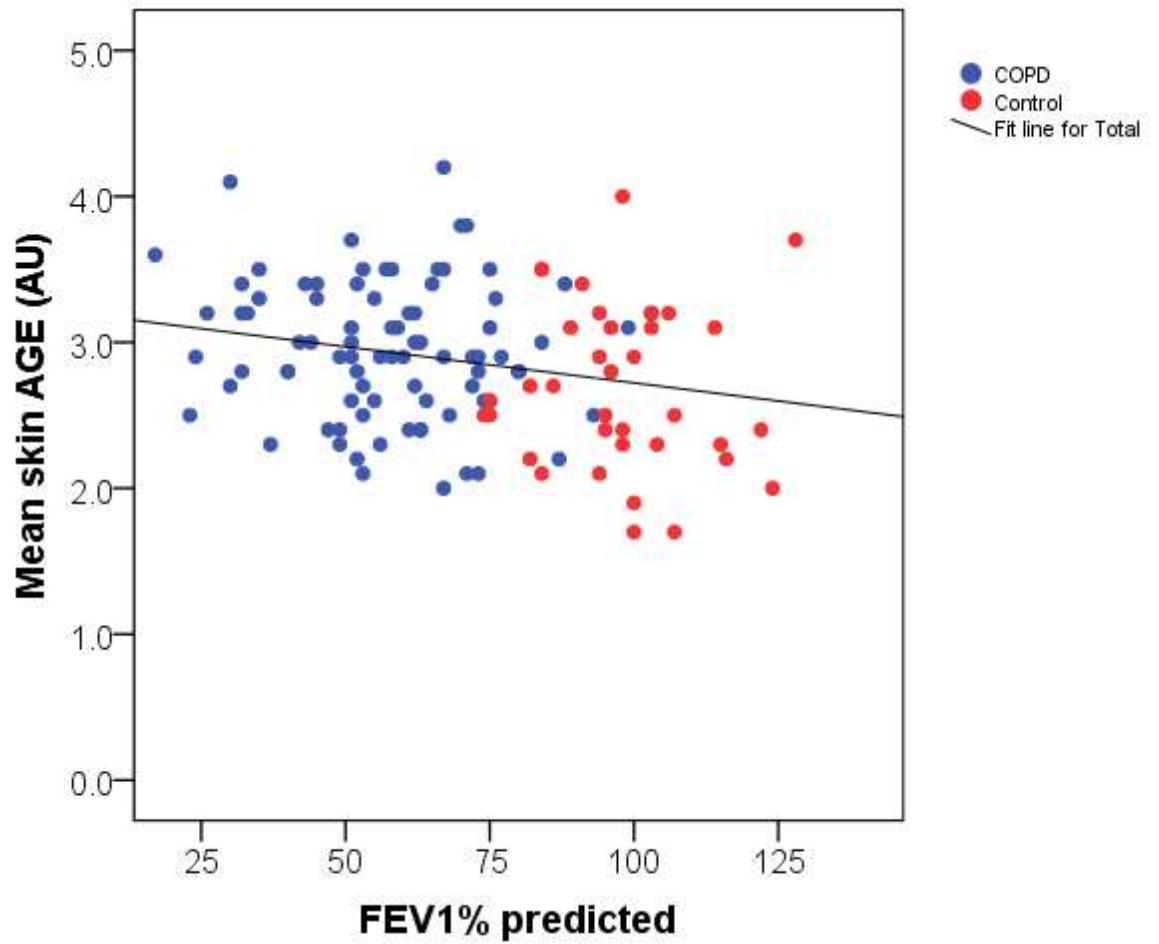
**Figure 6.3 Skin AGE Results Across GOLD Stages in Patients and Controls**



**Abbreviations – AGE:** advanced glycation end-products

The bars show the mean skin AGE for each of the GOLD categories and the control group; the error bars show 1SD.

**Figure 6.4 Skin AGE and FEV<sub>1</sub>% Predicted in Patients and Controls**



This figure shows the FEV<sub>1</sub> %predicted against mean skin AGE for the COPD and control groups, with a line of best fit across the whole group.

A stepwise multiple regression was performed in the COPD and control group, with skin AGE as the dependent variable and FEV<sub>1</sub>% predicted, smoking pack years, age, BMI and gender entered into the model. FEV<sub>1</sub>% predicted and age were independent variables  $p < 0.05$ , see Table 6.2.

When the same multiple regression analysis was performed in the COPD group alone, gender was the single independent variable,  $p < 0.05$ ; see Table 6.3.

In the total group FEV<sub>1</sub>% predicted was an independent variable of skin AGE over and above the effects of smoking pack years and age, and in the setting of similar BMI.

A priori, it was not our intention to statistically analyse the never smoker group in relation to the other smoker groups. Our never smokers were significantly younger. However, the relationship between the three groups is demonstrated in Figure 6.5.



**Table 6.2 Results of Significant Variables of Stepwise Multiple Regression in COPD Patients and Control group**

Dependent variable: skin AGE			
	<b>B</b>	<b>95% CI</b>	<b>Adjusted <math>r^2</math></b>
<b>Constant</b>	2.125	(1.45, 2.8)	
<b>FEV<sub>1</sub> % predicted</b>	-0.004	(-0.008, 0.000)	13.0%
<b>Age</b>	0.16	(0.007, 0.025)	9.4%

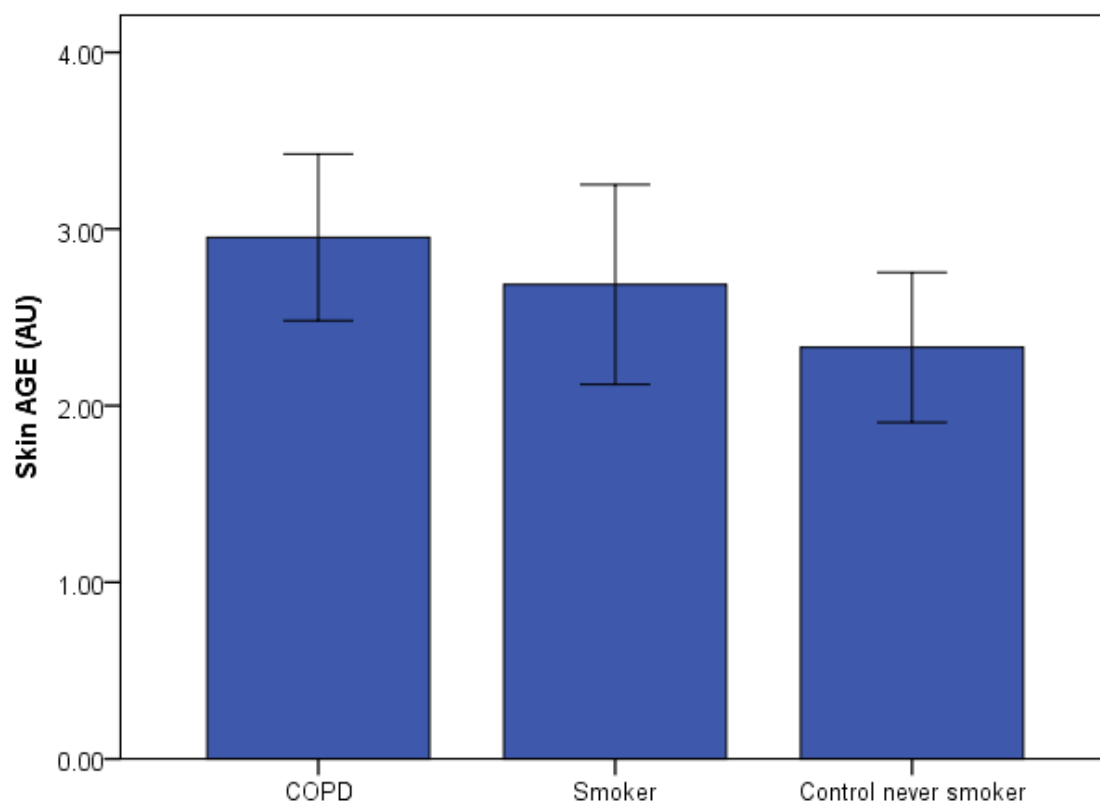
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**Table 6.3 Results of Significant Variables of Stepwise Multiple Regression in COPD Patients Alone**

Dependent variable: skin AGE			
	<b>B</b>	<b>95% CI</b>	<b>Adjusted <math>r^2</math></b>
<b>Constant</b>	3.355	(3.0.9, 3.635)	
<b>Gender</b>	-0.292	(-0.478, -0.074)	8.0%

See text p191 for variables entered.

**Figure 6.5 Skin AGE Levels Across All Groups**



**Abbreviations – AGE:** advanced glycation end-products

The bars represent the mean skin AGE and the error bars show 1 standard deviation.

### **6.3.2      Aortic PWV in Patients and Controls**

There was no significant difference in aortic PWV between the COPD (n=52) and control group (n=32) who had aortic PWV measurements,  $p=0.08$ , see Table 6.4; however, there was a trend towards increased aortic PWV in the COPD group. The subgroup who had aortic PWV were not different in age or sex compared to the total COPD and control group. There was no significant difference in aortic PWV across the different GOLD stages. There was no relation between aortic PWV and FEV<sub>1</sub>% predicted across the COPD and the control group, or in the COPD group alone ( $r=-0.101$ ,  $p=0.36$ ;  $r=0.111$ ,  $p=0.435$  respectively).

### **6.3.3      Other Haemodynamic Measures**

Mean arterial pressure (MAP) was increased in the controls compared to the patients, see Table 6.4.

There was no significant difference in brachial-femoral PWV between the COPD and control group,  $p=0.106$ , Table 6.4, or across the different GOLD stages. There was no relation of brachial-femoral PWV and FEV<sub>1</sub>% predicted across the COPD and control group, or in the COPD group alone.

There was no difference in mean Aix between the COPD and control group, Table 6.4.

### **6.3.4      Skin AGE and Haemodynamics**

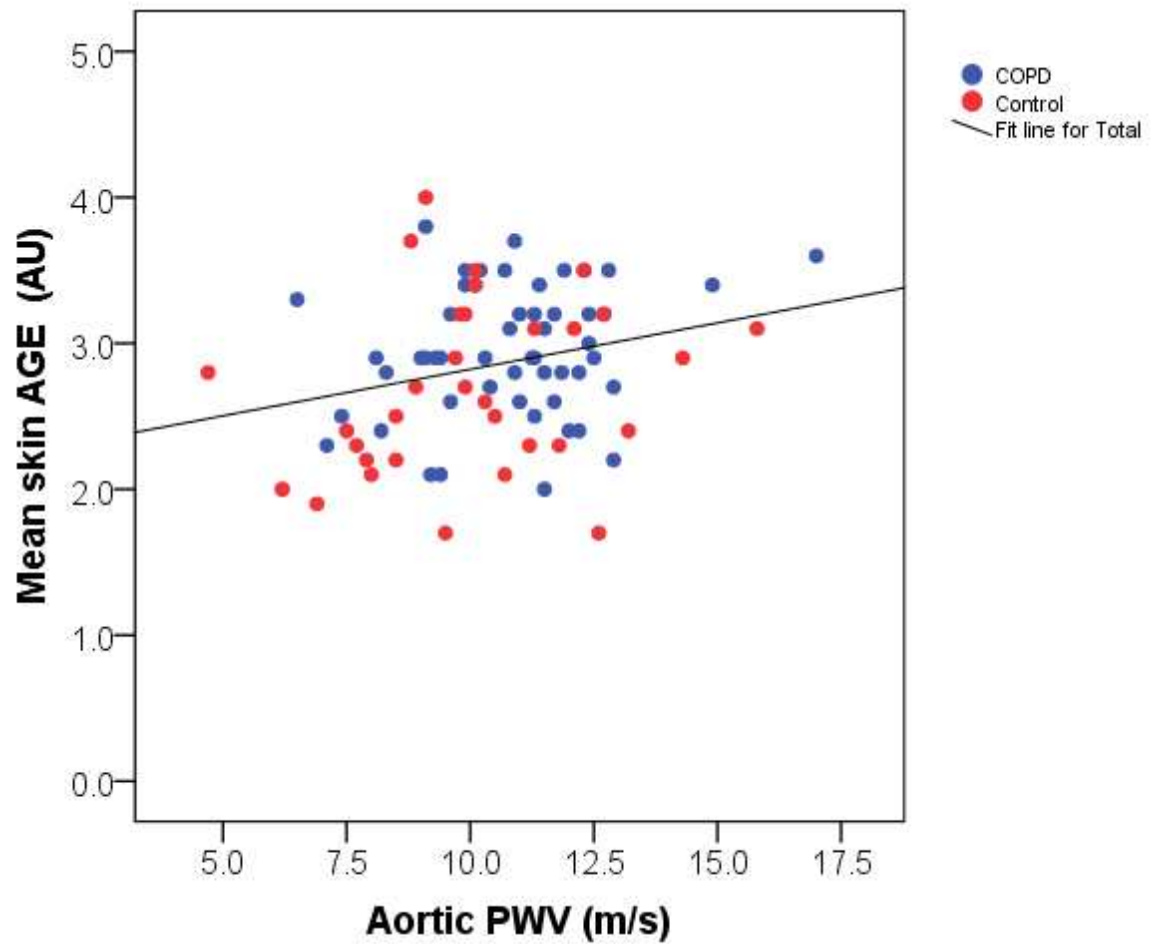
There was a direct relation between skin AGE and aortic PWV in the total group,  $r=0.26$ ,  $p<0.05$ ; see Figure 6.6. This was independent of age, gender and presence of comorbidities – self reported diabetes and CV disease. There was also a direct relation between skin AGE and brachial-femoral PWV in the total group,  $r=0.26$ ,

$p < 0.01$ ; which was again independent of age, gender and presence of comorbidities – self reported diabetes and CV disease.

In COPD alone there was a direct relation between skin AGE and brachial-femoral PWV,  $r = 0.24$ ,  $p < 0.05$ ; but no relation with aortic PWV,  $r = 0.22$ ,  $p = 0.13$ .

There was no correlation between Aix and skin AGE in the total group,  $r = -0.07$ ,  $p = 0.48$ ; or in the COPD group alone  $r = -0.08$ ,  $p = 0.46$ .

**Figure 6.6 Skin AGE and Aortic PWV in all Subjects**



**Abbreviations – AGE:** advanced glycation end-products, **PWV:** pulse wave velocity

Mean skin AGE (AU) and aortic PWV (m/s) correlation in COPD patients (n=52) and smoker controls (n=35).

**Table 6.4 Haemodynamic and AGE Results of Subjects Recruited to Each Group**

<b>Mean (SD)</b> Unless otherwise stated	<b>Controls</b> <b>n=36</b>	<b>COPD</b> <b>n=84</b>	<b>P values</b>	<b>Never smoker</b> <b>n=20</b>
<b>Aortic PWV (m/s) (n=52/35)</b>	10.0 (2.4)	10.8 (1.9)	0.088	9.0 (2.4)
<b>Brachial-femoral PWV (m/s)</b>	21.8 (8.2)	25.5 (14.7)	0.161	21.1 (11.4)
<b>Alx (%)</b>	21.0 (6.6)	22.2 (12.8)	0.599	20.9 (5.5)
<b>Peripheral systolic BP (mmHg)</b>	150 (22)	145 (20)	0.187	143 (21)
<b>Peripheral diastolic BP (mmHg)</b>	86 (12)	83 (11)	0.263	87 (11)
<b>Peripheral PP (mmHg)</b>	64 (17)	61 (17)	0.382	56 (14)
<b>MAP (mmHg)</b>	114 (15)	108 (15)	0.048	110 (13)
<b>Heart Rate (bpm)</b>	71 (11)	77 (15)	0.025	70 (12)
<b>Skin AGE (AU)</b>	2.7 (0.6)	3.0 (0.5)	0.009	2.3 (0.4)
<b>Serum AGE<sub>t</sub> (pg/ml)</b> (n=34/54/14)	4612.1 (2.4)	4525.8 (2.1)	0.912	3865.4 (2.3)
<b>Serum soluble RAGE<sub>t</sub> (pg/ml)</b>	1017.0 (1.7)	793.6 (1.5)	0.006	1130.0 (1.5)

**Key** - <sub>t</sub> = geometric mean (SD)

**Abbreviations-** **PWV**: pulse wave velocity, **Alx**: Augmentation index, **BP**: blood pressure, **PP**: pulse pressure, **MAP**: mean arterial pressure, **AGE**: advanced Glycation End-products, **RAGE**: receptor for AGE.

### **6.3.5      Skin AGE and Comorbidities**

#### *6.3.5.a   Diabetes*

When those subjects with self-reported diabetes (n=13) were compared to those without diabetes (n=107) there was no significant difference in skin AGE, 3.1 (0.4)AU, 2.8 (0.5)AU respectively, p=0.06.

A sub-group analysis was performed in COPD patients alone, where those with self-reported diabetes (n=9) were compared to those without diabetes (n=75), 3.0 (0.4)AU and 2.9 (0.5)AU respectively, and there was again no significant difference in skin AGE, p=0.6.

#### *6.3.5.b   IHD*

When those subjects with self-reported IHD (n=15) were compared to those without IHD (n=105) there was no significant difference in skin AGE, 3.0 (0.5) and 2.9 (0.5) respectively; p=0.28.

A sub-group analysis was performed in COPD patients alone, where those with self-reported IHD (n=12) were compared to those without IHD (n=72) and there was again no significant difference in skin AGE, 3.0 (0.5) and 2.9 (0.5) respectively; p=0.4.

### **6.3.6      Serum AGE and RAGE**

There was no difference in circulating AGE between the patients with COPD and the control group, see Table 6.4; but we were able to detect a significant difference in soluble RAGE - patients with COPD 793.6 (1.5)pg/ml and controls 1017.0 (1.5), p=0.006, see Table 6.4.

There was a direct relation between  $\log_{10}$  RAGE and both FEV<sub>1</sub>% predicted and FEV<sub>1</sub>/FVC,  $r=0.24$ ,  $p<0.05$  and  $r=0.34$ ,  $p<0.01$  respectively in the COPD patients.

There was no correlation between sRAGE and aortic PWV,  $r=-0.26$ ,  $p=0.18$  in the total group and  $r=-0.30$ ,  $p=0.30$  in COPD alone.

### **6.3.7 Inter-operator Validation of PWV**

Bland Altman Plots were used to look at aortic and brachial-femoral arterial stiffness and both sitting and supine augmentation index in the 10 patients, see Figure 6.7. There was little variability between the two operators for aortic PWV, brachial-femoral PWV and sitting and supine Alx, with the majority of measurements being within 2SD.

As all 4 plots have the data clustered around the 0 line there is no evidence of proportional error; instead systematic error is shown.

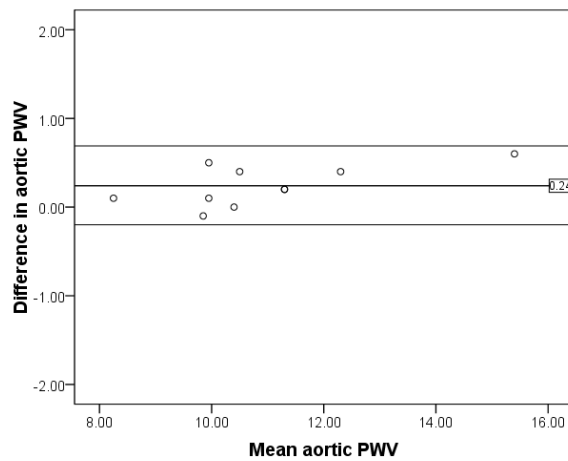
Systematic error is an error arising from the measuring instrument, often as a result of a fault or incorrect use; thus the systematic error could have arisen by the measurements of path length being different by the two operators.

Bland Altman Plots have also been done to look at the same blood pressure results from both members of staff performing measurements for the study, see Figure 6.8. There was little variability between sitting and supine systolic and diastolic blood pressure measurements for both operators with all results within 2SD, adding further support that there was no significant difference in measurements performed by either staff member.

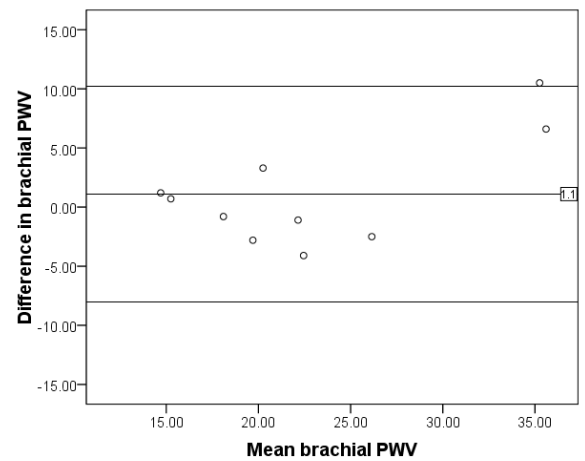


**Figure 6.7 Bland Altman Plots of Haemodynamic Measurements Between 2 Operators**

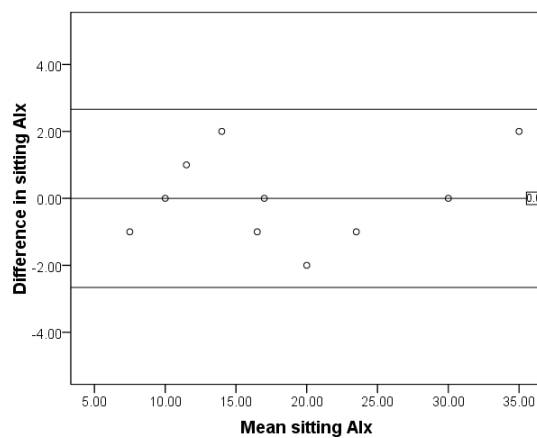
**a. Aortic PWV**



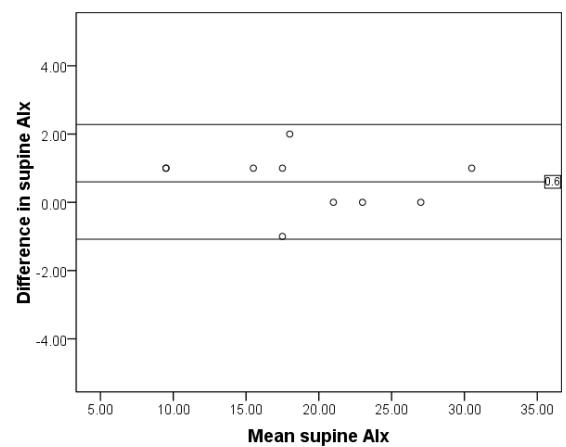
**b. Brachial-femoral PWV**



**c. Sitting Alx**



**d. Supine Alx**

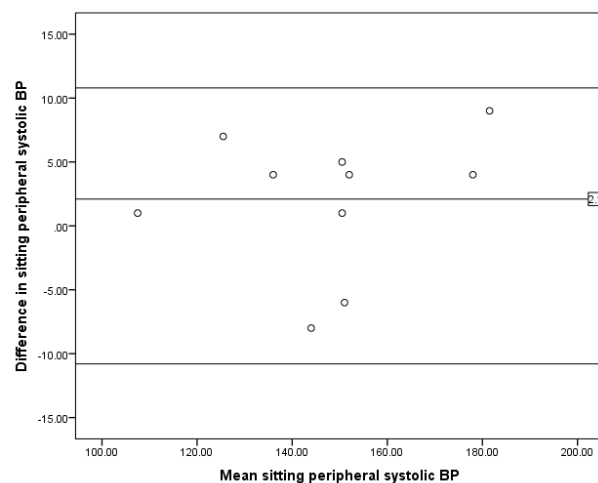


**Abbreviations – PWV:** pulse wave velocity, **Alx:** augmentation index

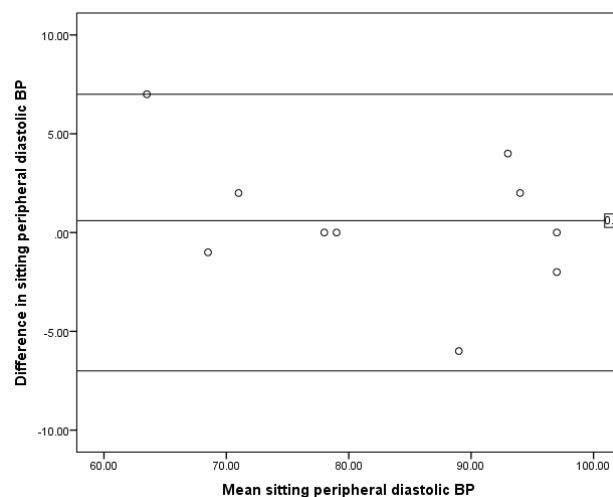
The lines represent the mean and  $\pm 2$  standard deviations.

**Figure 6.8 Bland Altman Plots of Blood Pressure Measurements Between 2 Operators**

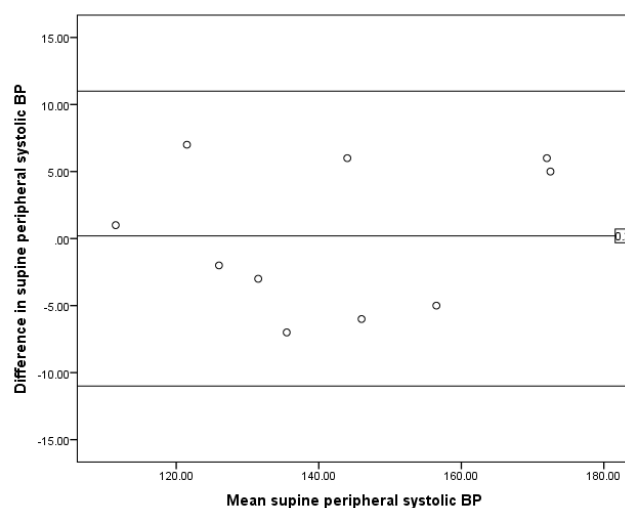
**a. Sitting systolic BP**



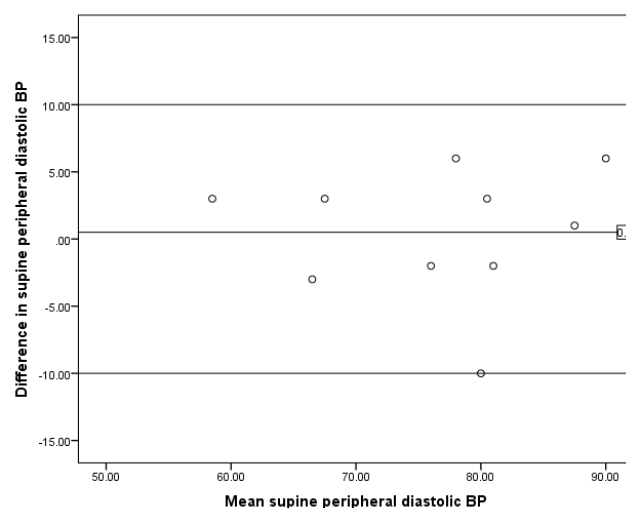
**b. Sitting diastolic BP**



**c. Supine systolic BP**



**d. Supine diastolic BP**



**Abbreviations – BP: blood pressure**

### 6.3.8 Validation of AGE Reader

Repeated sets of AGE readings were made on 20 different subjects; see Table 6.5. In 20 subjects two sets of measurements were made consecutively. On 11 of these subjects an additional set of measurements were made between 24 hours and 1 week later (measurement 3) – see Figure 6.9.

**Table 6.5 Repeated Skin AGE Results**

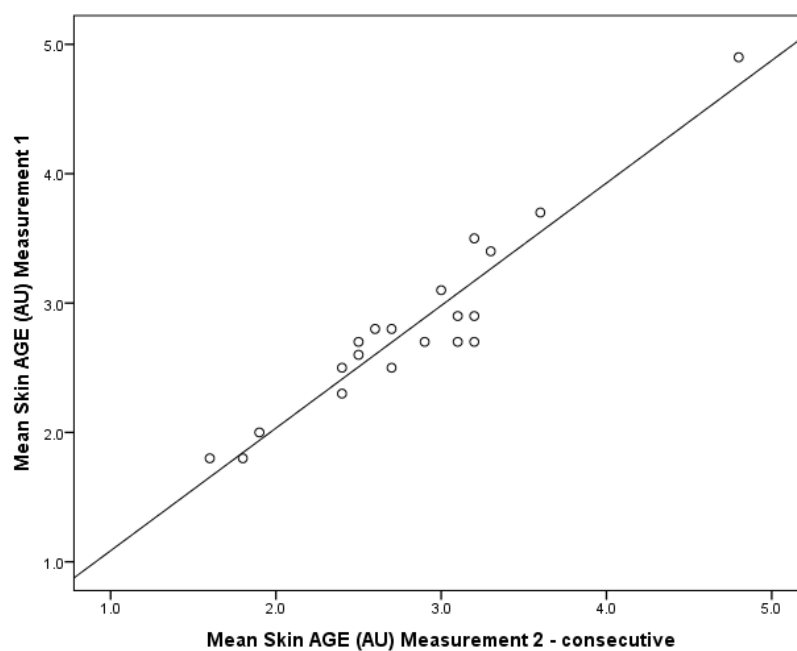
	<b>Measurement 1 n=20</b>	<b>Measurement 2 Consecutive n=20</b>	<b>Measurement 3 &gt;24hrs later n=11</b>
<b>Skin AGE (AU)</b> range	1.8 - 4.9	1.6 – 4.8	1.8-4.6
<b>Skin AGE (AU)</b> mean (SD)	2.8(0.7)	2.8 (0.7)	2.9 (0.71)

**Abbreviations** – AGE: advanced glycation endproduct, AF: autofluorescence

A coefficient of variation analysis was used to test for mean skin AGE differences among three different measurements, to see if there was any difference in AGE readings made at different time points. Mean skin AGE did not differ significantly across the three measurements, coefficient of variation was less than 10% in all cases, with a mean coefficient of variation of 4.6% demonstrating the skin AGE measurements are repeatable over time.

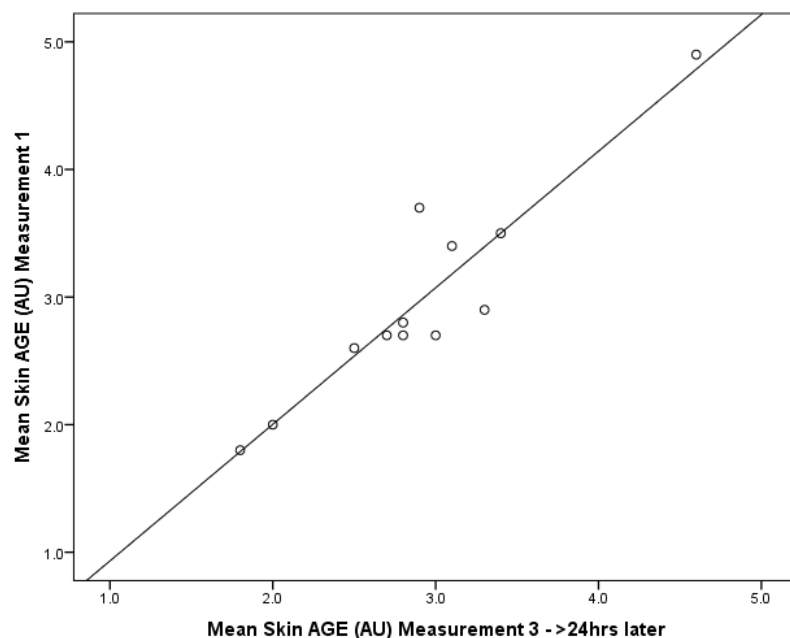
**Figure 6.9 Mean Skin AGE Correlation Plots**

**a) Correlation between consecutive skin AGE measurements**



Correlation between 2 consecutive measurements of mean skin AGE (AU) (n=20).

**b) Correlation between mean skin AGE measurements made >24hrs apart**



Correlation between 2 mean skin AGE (AU) made at least 24 hours apart (n=11).

**Abbreviations – AGE** : advanced glycation end-products

## **6.4 Discussion**

Skin AGE using skin autofluorescence were increased in clinically stable patients with COPD compared to smoker controls, were inversely related to lung function and directly related to aortic stiffness in all subjects, but not in COPD alone, comparable to previously reported results in patients with moderate to severe COPD (Gopal et al., 2013).

### **6.4.1 Lung Function and Skin AGE**

The relationship between reduced lung function and cardiovascular risk has been well documented (Sin et al., 2005). We have shown that increased skin AGE is related to a lower lung function however this was only seen in the total population and not in the COPD group alone. Consistent findings were seen in a study conducted in The Netherlands, where an inverse relationship between skin AGE and lung function was reported in the main group of patients with COPD and controls, but not in the COPD patients alone (Gopal et al., 2014).

### **6.4.2 Haemodynamic Measurements and Skin AGE**

It has previously been shown that serum AGE levels are associated with aortic PWV in the general population (Semba et al., 2009b) and in those with renal disease (Ueno et al., 2008). We have shown a similar association with aortic PWV using skin AGE in patients with COPD.

Given the age of the population in this study it would be expected that the relationship between skin AGE and aortic stiffness seen would be with aortic PWV as opposed to Aix (Mitchell et al., 2010). This is due to the differing nonlinear

dependency on aortic wall stiffness with PWV and pressure pulsatility, and the discordant change with age (Mitchell et al., 2004). The common risk factors for CV disease and COPD have been previously reported (Barnes and Celli, 2009), but the association remains over and above this.

Using the same vicorder machine to measure aortic PWV a study has recently published results of 8.9m/s in a COPD population of 23 subjects (Stone et al., 2013) which, despite using the same technique, are lower results than we found in our population. There are no obvious reasons why the aortic PWV reported by Stone *et al.* should be different to that found in this study, as baseline lung function, smoking history, age and BMI is comparable, with both populations having a higher proportion of males; similar to us, they did not exclude those with a cardiac history or cardiac risk factors, but they recruited from a COPD and CV database, therefore there could be a potential disparity between CV disease prevalence in the study group and current medications.

#### **6.4.3      AGE and Comorbidities**

Many of the common co-morbidities in patients with COPD, such as atherosclerosis (Wautier and Schmidt, 2004), osteoporosis (Yamagishi et al., 2005), and diabetes (Nin et al., 2011) have been associated with increased AGE levels in their own right. The increased CV risk in diabetics has been hypothesised to be due to the increased circulating AGE levels, and therefore a similar process may help to explain the CV complications in patients with COPD. A correlation between sRAGE and CV disease severity has previously been reported in a Chinese population with coronary artery disease (Lu et al., 2011).

The population in our study was inclusive of co-morbidities, and had few exclusion criteria. There was no difference in skin AGE in either the total group or just the COPD group, between those with IHD and those without IHD, or between those with diabetes and without diabetes; however, there was a disparity between numbers. Our IHD and diabetes record was self-reported and encompasses a wide timeframe and range of presentations – for example, we were not able to distinguish between those with a previous MI or current angina, likewise we could not distinguish between those with diet controlled diabetes or end-stage diabetes. This generic group reflects a typical population as seen in a clinic setting and therefore makes the results more representative. It also means that if patients presented with subclinical changes, excluding a subset with a prior diagnosis is arbitrary, and may relate to a diagnosis as opposed to presence bias.

#### **6.4.4      Circulating AGE in Patients with COPD**

Since starting this project, circulating plasma N-(carboxyethyl)lysine (CEL), a well characterised AGE, has been reported as increased in patients with COPD compared to never/ex-smoker controls (Gopal et al., 2013, Gopal et al., 2014). The COPD population these results were reported in were GOLD stage 2,3 and 4; n=34, n=39 and n=15 respectively, similar to our population where the majority were GOLD stage 2-3; however, we were unable to detect any significant difference in AGE levels. There are several possible explanations for the increased formation and levels of systemic AGE including chronic inflammation and oxidative stress (Gopal et al., 2013). The accumulation of AGE is also heightened by hyperglycaemia and decreased renal clearance of AGE precursors (Miyata et al., 1997).

#### **6.4.5      RAGE in Patients with COPD**

Different studies measure circulating sRAGE through different methods. The ECLIPSE study – a longitudinal observational study measured circulating sRAGE in serum samples, comparable to this study, and found sRAGE levels were significantly and inversely associated with disease severity as defined using GOLD stages in 2,349 subjects from 46 centres in 12 countries (Cheng et al., 2013). Alternatively, the Treatment of Emphysema With a Gamma-Selective Retinoid Agonist study (TESRA) which was a phase 2, double-blind, placebo controlled RCT with over 400 patients with COPD measured sRAGE in baseline plasma samples and reported no significant difference in sRAGE between GOLD stages 2 and 3 (Cheng et al., 2013), however, this study was in a predefined group of emphysema patients. It has previously been suggested that RAGE cause structural vascular wall changes (Basta, 2008).

#### **6.4.6      Lung Function and RAGE**

RAGE has been shown to be significantly increased in COPD patients compared to controls, with a rise in mean sRAGE with increasing GOLD stages (Gopal et al., 2013), and although our study showed a similar trend we did not reach significance.

#### **6.4.7      Repeatability and Validation**

##### *6.4.7.a Haemodynamic measurements*

The vicorder is a technically easier method to perform compared to other methods of measuring arterial stiffness, and it is now becoming a more widely accepted method of determining aortic PWV. During a small validation study there were no differences between 2 operators performing measurements on the same subjects,



showing the test is not operator dependent. Recently published studies have shown aortic PWV using a vicorder to be highly reproducible when used in patients with COPD (Stone et al., 2013), and in the general population (McGreevy et al., 2013).

#### *6.4.7.b Skin AGE measurements*

There was good repeatability and no significant inter-observer differences showing the AGE reader was consistent over time and did not have significant day to day fluctuations.

### **6.4.8 Future Utility of Skin AGE**

The recent GOLD guidelines highlight the need to actively look for comorbidities in COPD patients (Decramer et al., 2013). Skin AGE measurements are a potential tool that can be easily implemented in a clinical setting to help identify patients that are at risk of comorbidities. Based on this pilot work there are potential clinical applications for skin AGE measurements, given the relationship with skin AGE and FEV<sub>1</sub>% predicted and aortic PWV the skin AGE reading may be able to help identify patients at risk of CV disease and lung function decline; however, only longitudinal studies will be able to fully explore this. This is of particular relevance as GOLD highlights CV disease as being frequent and important (Decramer et al., 2013).

Skin AGE has greater utility when compared to the measurements of circulating AGE levels, and has been validated against the GOLD standard skin biopsy measurements (Meerwaldt et al., 2004). When skin AGE measurements are performed the results are available immediately. It is non-invasive and easy to perform, requiring little effort from the subject. Skin AGE results reflect the

accumulation in the body, compared to circulating AGE levels which fluctuate with diet (Vlassara et al., 2002) and smoking (Cerami et al., 1997). Circulating AGE and RAGE levels require a blood sample to be taken, a specialised analysis technique, and therefore a subsequent delay time for results.

#### **6.4.9      Limitations and Points for Consideration**

Limitations to the study include the difference in smoking history between the COPD and control groups of 46 and 28 pack years respectively. However, this does reflect the nature of COPD and its association with a significant pack year smoking history. Further, all subjects had a greater than 10 pack year smoking history and this was adjusted for in regression analysis. All subjects were of European ancestry, as required for the skin AGE reading, and possible effect on haemodynamic results (Meerwaldt et al., 2004). Aortic PWV was only available in a smaller number of subjects, and not in the total study population.

Restrictions to the utility of the skin AGE reader are that the machine is only validated for use in Caucasian subjects. The use of steroids and their effect on skin AGE measurements has been questioned; however, research looking at other chronic inflammatory disease population has not found any effects with steroid use and skin AGE measurements (de Leeuw et al., 2007), and more recently no effect on skin AGE was found with either oral steroids or statins in a COPD population (Gopal et al., 2013). Prospective, longitudinal studies are needed to comprehensively evaluate the potential influencing factors, such as co-morbidities, involved in changes in circulating and skin AGEs in patients with COPD.

#### **6.4.10      Conclusion**

There is evidence of increased skin AGE levels in COPD patients compared to controls; and a relationship between skin AGE and airways obstruction, and skin AGE and aortic PWV. It is possible that AGE is a linking mechanism between lung function and arterial stiffness. Skin AGE measurements may represent a future diagnostic tool that can be implemented in a clinical setting to aid the identification of those at risk of comorbidities, particularly CV disease, in accordance with the GOLD guidelines; and further studies are warranted.

# **Chapter 7**

## **Conclusions**

## **7 Conclusions**

### **7.1 Rationale for the Studies in this Thesis**

There is an increasing awareness of the development of extra-pulmonary co-morbidities in COPD, to the extent that it is now incorporated into the GOLD strategy and international definition of COPD. These extra-pulmonary effects include CV disease and have been shown to have an adverse effect on morbidity and mortality.

There is a large gap in therapy options for these extra-pulmonary manifestations and research to explore alternatives and attenuate such risk is needed. In parallel, there is a need to identify better biomarkers of the disease, new therapies that might address this and how biomarkers alter with therapy.

Statins are a group of lipid lowering drugs which have been shown in both primary and secondary prevention to reduce CV mortality and morbidity in selected patients with normal or elevated cholesterol levels.

It was proposed that statin therapy in COPD, through modulating systemic inflammation and lipid lowering effects may favourably modify non-invasive markers of CV disease in the short term. This thesis comprises a randomised, placebo controlled, double blind trial investigating the CV and inflammatory effects of statins in COPD, and a cross-sectional study investigating the associations between CV risk and COPD.

## 7.2 Conclusions

### 7.2.1 Cardiovascular Effects of Statin Therapy in Patients with COPD

A well-defined group of clinically stable patients with COPD, without hypercholesterolaemia, known diabetes or IHD, were randomised 1:1 to either simvastatin 20mg or placebo for 6 weeks treatment. Despite a significant reduction in total cholesterol there was a non-significant improvement in our primary endpoint - aortic stiffness, as measured by aortic PWV – an independent predictor of CV risk, in patients with COPD taking simvastatin 20mg compared to placebo over 6 weeks. There were no significant changes post-treatment in any of the other haemodynamic measurements including blood pressure, MAP and Aix.

Our baseline aortic PWV was lower than we were expecting based on reported values at the study set-up, however, the results are in keeping with recently published results which are still greater in patients with COPD than in controls. We also reported wider SD than what the power calculation was based on. This has led the study to be underpowered to detect the change we set out to see. We did report a 1m/s drop in aortic PWV in the active group as we set-out to do, but due to the placebo effect the difference between active and placebo group was not significant.

In a pre-defined subgroup with a baseline aortic PWV value of >10m/s we did demonstrate a significant change in aortic PWV post-treatment in the active compared to placebo group. This was in parallel to a significant drop in total cholesterol post-treatment in the active compared to the placebo group. Therefore, subjects with a higher baseline aortic PWV may be likely to have more

benefit from intervention. In line with the main group analysis there was no significant change in any of the other haemodynamic measurements post treatment in the active compared to placebo group.

### **7.2.2 Inflammatory Effects of Statin Therapy in Patients with COPD**

In this RCT, there was not a detectable change in either airway or circulating inflammatory markers. We were unable to detect any change in airway inflammatory markers of exhaled nitric oxide (FeNO), induced sputum – neutrophils or macrophages; or circulating markers – hs-CRP and total MMP-9, post treatment in the active compared to placebo group following 6 weeks treatment with active compared to placebo. Of importance, airway inflammatory markers were only available in a limited number of subjects.

In a sub-group analysis of patients with a baseline hs-CRP >3mg/l, n=33, 16 active and 17 placebo, there were no significant changes in hs-CRP post-treatment in the active compared to placebo group. In addition, there were no changes in airway inflammatory markers or total MMP-9 in this sub-group.

### **7.2.3 Relationship of Haemodynamics to Inflammatory Measures in Patients with COPD**

There were no demonstrable associations between airway or systemic inflammatory markers and aortic stiffness in a group of well-defined patients with COPD, without hypercholesterolaemia, IHD or diabetes. Although there was a trend towards higher hs-CRP in those in the lowest aortic PWV group this did not

reach significance. Using a variety of pre-defined cut-off points and tertile analysis did not allow any relationships to be detected.

#### **7.2.4      Advanced Glycation End-products (AGE) and CV Risk in Patients with COPD**

Skin AGE levels have been validated against skin biopsies and provide a reliable, non-invasive method of assessing AGE levels as a potential biomarker of risk of comorbidity patients with COPD. We were able to report increased skin AGE levels in COPD patients compared to controls; and a relationship between skin AGE and FEV<sub>1</sub>% predicted and skin AGE and aortic PWV in the total group but not in COPD alone. Skin AGE measurements may be a potential future diagnostic tool for use in a clinical setting to aid the identification of those at risk of comorbidities, particularly cardiovascular disease, in accordance with the GOLD guidelines requirements. However, larger studies are required, and ideally would include a longitudinal element.

### **7.3 Summary**

There are numerous co-morbidities seen in COPD and in this thesis I have focused on CV disease with statins as a potential therapy to attenuate the CV implications, and attention to biomarkers to detect this risk in patients with COPD. Although we were not able to show a significant difference in the change in aortic PWV between those taking the active statin treatment compared to placebo post-treatment; we did find a significant change in those with a baseline aortic PWV >10m/s and therefore treatment may be better aimed at those with a baseline aortic PWV >10m/s and warrants further investigation. I have also explored the role of skin



AGE as a potential new biomarker, and the possible association between AGE and arterial stiffness. This is an expanding area of research and one with important consequence, given the increasing prevalence of COPD and the projection for it to be the third leading cause of death by 2020 having considerable impact on morbidity and mortality, quality of life and the economic burden. These results highlight the need for further studies in high risk patients to confirm the impact of statin use on the CV outcome of COPD, using death as primary outcome; and give an indication of their potential role in this sub-group of patients.

# **Chapter 8**

## **Future directions**

## **8 Future Research Directions**

In order to build upon the research work presented in this thesis there are several other areas of research that I feel would be particularly pertinent. Further studies in high risk patients with COPD are needed to confirm the impact of statin use on the cardiovascular outcomes. There are 3 approaches that could be explored.

- The first is a 'hard endpoints' study looking at CV morbidity and/or mortality. The results of this RCT may lead onto larger, more pragmatic studies with morbidity outcomes as an endpoint in patients with COPD, irrespective of lipid levels. Having studied a specific population to deliberately avoid patients who would be eligible for statin treatment based on current licensed indications, we cannot generalise our findings to the total population of patients with COPD. A study examining the wider COPD population would be more representative of patients presenting in a clinical setting. However these are costly, of long duration and require some background supporting information such as this proof of principle RCT.
- The second approach is a study looking at aortic PWV in either a greater number of people, or in those with increased baseline aortic PWV - as we have shown a potential benefit in subject with a baseline aortic PWV >10m/s, a study looking at this population with a pre-defined increased aortic PWV could be performed; but given our recruitment difficulties this would need to be a multi-centre study to allow recruitment targets to be met. Alternatively these study options could be performed in 'all' COPD subjects, therefore including those with CV disease but not on

statins already, as this would then be a more representative sample of patients with COPD and would be easier to recruit to.

- This was a pilot study looking at feasibility for future studies. Any intervention that may reduce the risk of CV disease in COPD requires careful evaluation. We selected an intervention that lasted for 6 weeks, comparable to timeframes used in previously published work, and also for patient retention. We are unable to exclude the possibility that a longer interventional timeframe or a higher dose of statin may have a beneficial effect on modifying aortic stiffness. Further research looking at the effect of dose and duration of statin use in patients with COPD should be considered.
- A third way to build on the research presented in this study is through an epidemiological approach, although this would not take the place of the first two clinical study options. Using a large database, for example the GP health improvement network (THIN) dataset it could be assessed whether CV profiling is routinely performed on patients with COPD, because COPD management does not currently include a routine CV assessment or any primary prevention, despite the known increased CV risk. The data can be used to look at the proportion of patients with COPD that are on a statin and determine whether statin therapy reduces total mortality and CV mortality using cox regression analysis. However, any such results would be limited by their speculative nature, as they would be based on retrospective cohort analysis and therefore liable to confounding bias.

In parallel, there is a need for a major shift in the care of patients with COPD to address the cardiovascular risk at diagnosis and at assessments. Known risk factors can be addressed such as smoking cessation and treating hypertension and hypercholesterolaemia. Primary prevention and other therapeutic interventions to supplement these endeavours should be prioritised.

At COPD diagnosis and follow-up assessments, there is not a comprehensive review of CV risk. To build on the work looking at skin Advanced Glycation End-products as a potential new biomarker to detect those patients with COPD most at risk of CV comorbidity, prospective, larger, longitudinal studies are needed to fully evaluate potential influencing factors, including co-morbidities which are involved in changes in circulating and skin AGEs in patients with COPD. However, this should be done in parallel with risk score calculations (e.g. Framingham, National Heart Lung and Blood Institute (NHLBI)) to see if the skin AGE offers additional benefit to these traditional calculators, as the skin AGE reader machine is costly. Given that the risk of CV disease and death is over and above the risk in smokers in epidemiological studies, one could imagine that the risk score calculators might not identify the risk in patients with COPD, as the current risk score calculator algorithms centre on the modifiable factors of blood pressure, lipids and smoking. However, the risk scores have not been fully researched and subtle changes in the parameters might account for any differences. Alternatively, a COPD modified calculator might be necessary.

## 9 Appendices

**Patient Information Sheet** Version 1.3 02-03-10

**The Cardiovascular and Inflammatory Effects of Statin Therapy in Patients with  
COPD**

**Chief Investigator: Dr Charlotte Bolton**

**REC ref:** 10/H0408/10

**Introduction**

We would like to invite you to take part in our research study. It is important for you to understand why the research is being done and what it would involve for you, before you decide whether or not you would like to take part. One of our team will go through the information sheet with you and answer any questions you have. Please take time to read this information sheet carefully and talk to others – family or friends, about the study if you wish. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

**Part 1 of the information sheet**

**What is the purpose of the study?**

The research is looking at whether a drug called simvastatin could be used as an additional treatment for people with chronic obstructive pulmonary disease.

Chronic obstructive pulmonary disease (COPD) is a condition of the lungs which results in breathing difficulties. This is due to the lungs being damaged. The lungs become inflamed and the breathing tubes (airways) narrowed. In addition, we know there is increased inflammation in the blood and patients with COPD are at increased risk of heart disease. Current treatments have focused on the narrowed airways and attempt to open them up. Statins, simvastatin being one of them, are drugs used to lower cholesterol in the blood. In addition, it may also reduce inflammation and lower the risk of heart disease. This study will explore whether simvastatin has a benefit in patients with COPD.

### **Why have I been invited?**

You have been invited to participate because you have COPD and you

- Have previously consented to be included on the BRU respiratory database
- Are attending one of the researchers' clinics
- Have replied to one of the posters seeking volunteers

### **Do I have to take part?**

It is entirely up to you whether or not you decide to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form (you will be given a copy of this as well). If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw, or a decision not to take part, will not affect the standard of care you receive.



## **What will happen to me if I take part?**

The study involves taking part in a study to explore the effects of simvastatin. It is a randomised placebo controlled study which means we will give study participants either a 6 week course of simvastatin, or a 6 week course of placebo (a dummy treatment which looks like the genuine medicine but contains no active ingredient) and then compare the effects of the two treatments at the end. Sometimes we don't know which way of treating patients is best. To find out, we need to compare different treatments – in this study we are comparing simvastatin to a placebo. To try and make the study as fair as possible each participant will have an equal chance of getting simvastatin or placebo, and the trial will be double-blind which means neither you nor the people running the study will know which treatment you are taking (although if need be we can find this out). The placebo (dummy treatment) contains lactose (sugar found in milk), so you will be unsuitable if you are lactose intolerant.

If after reading this information sheet and talking to a member of the research team you agree to take part in this study, there will be 3 visits to the Respiratory Biomedical Research Unit (BRU) in the Clinical Sciences Building at City hospital. We will ask for your consent in writing first.

### Consent Visit

If you agree to take part in the study you will need to sign an informed consent sheet so that we have written evidence of your wish to participate in the study.

### Screening visit

Please refrain from taking your inhalers before this visit. Your reliever inhalers (usually blue or grey) should be not used for 6 hours before your visit. Long acting inhalers (ones you take just morning and night) should not be taken for 12 hours before your visit. Please fast for 8 hours before coming to this visit – including drinks containing caffeine. Please do not smoke for 6 hours before coming to this visit.

At the screening visit we will do the following:

- Take a full medical history including smoking history, and medical examination
- Perform spirometry (a simple breathing test where you blow hard and fast into a tube for as long as you can). Depending on your results we may ask you to take an inhaler (salbutamol) and repeat the breathing test again so that we can measure any changes in your airways – this is called a reversibility test
- Measure the level of a gas called carbon monoxide exhaled in your breath. This involves blowing into a machine which is a bit like an alcohol breathalyser
- Take a 10ml blood sample (about 2 teaspoons) – this is required as part of the safety monitoring of the trial. We will measure your blood count, kidney and liver function and fasting lipids (e.g. cholesterol). If you are a female and there is a possibility you might be pregnant, we will also do a pregnancy test as the drug is not indicated in pregnancy.
- Capillary blood gas sample – a small scratch on your earlobe to take a few drops of blood to measure your oxygen and carbon dioxide levels

There will then be a period of about 2 weeks whilst we wait for all your test results.

### Visit 1

Please refrain from taking your inhalers before this visit. Your reliever inhalers (usually blue or grey) should be used for 6 hours before your visit. Long acting inhalers (ones you take just morning and night) should not be taken for 12 hours before your visit. Please fast for 8 hours before coming to this visit – including drinks containing caffeine. Please do not smoke for 6 hours before coming to this visit.

During visit 1 we will do the following:

- Measure your height and weight. In addition we will measure the amount of fat and muscle in your body using special weighing scales.
- Issue of study drug (simvastatin) or placebo
- Take a blood sample (25mls which is about 5 teaspoons) to measure inflammatory markers, liver function, a muscle marker and markers of kidney and heart function. In addition we will measure lipid levels (e.g. cholesterol). Some of these tests are clinically relevant, some are to aid us in research understanding.
- Spirometry breathing test
- Take a sputum sample – to measure the inflammatory cells in the sputum. If you cannot produce sputum readily we can ‘induce sputum’ by asking you to inhale a salt solution called hypertonic saline
- Measure the level of a gas called nitric oxide exhaled in your breath. This involves blowing into a machine which is a bit like an alcohol breathalyser

- Measure the level of a gas called carbon monoxide exhaled in your breath. This involves blowing into a machine which is a bit like an alcohol breathalyser
- Measure blood pressure
- We will then measure artery stiffness by placing an electronic pencil shaped device lightly over the artery pulse in your wrist. Following this, you will be asked to lie on a bed for 20 minutes where we will take readings from the arteries at the wrist (radial), neck (carotid) and upper thigh (femoral).
- A walking test – to see how far you can walk in 6 minutes. Before we do this, we can let you have a light snack.
- Measure handgrip strength
- Complete a breathlessness and quality of life score questionnaire
- Provide a urine sample – about 10mls (2 teaspoon)

In between the 2 visits you will receive 2 telephone calls to see how you are getting on and remind you of any forthcoming appointments.

## Visit 2

During visit 2 we will do the same as we did in visit 1. This will allow us to compare your results before and after your 6 weeks on simvastatin or placebo.

## **Travel Allowance**

We can reimburse travel costs associated with coming to the BRU. Please keep receipts.

**What will I have to do?**

We ask that you take the study medication regularly as directed. You can continue to take your other normal medication which will be checked at the screening visit to make sure it will not affect the study medication. It is important that you are not involved in any other drug studies whilst taking part in this study. Please let us know if you have taken part in another drug study within the last year. We also ask that you attend the scheduled follow-up study visit – there is some flexibility in terms of the day and time when this is scheduled.

**What is the drug, device or procedure that is being tested?**

Simvastatin is a tablet that is commonly used to lower cholesterol in the blood. It will be taken by mouth at a dose of 20mg once a day in the evening for 6 weeks.

Please let your doctor or pharmacist know that you are taking part in this study if a change to your normal treatment is made while you are taking your study medication.

There are a number of drugs that can interact with simvastatin. If you agree to participate we will check your current medication is satisfactory and will not cause any interactions. Such drugs include other cholesterol lowering drugs, an antibiotic – clarithromycin; but also grapefruit juice.

**What are the alternatives for diagnosis or treatment?**

The alternative is to continue with your current treatment.

**What are the side effects of any treatment received when taking part?**

Simvastatin is a treatment commonly used to treat people with high cholesterol. As with all medicines some people develop side effects after taking simvastatin. The main side effects that have been reported by people taking simvastatin are muscular effects such as aching muscles, tender muscles or muscle weakness which are rare but often significant. Statins can also affect liver function blood tests. Rarely they can cause yellow jaundice and headache.

If you are concerned that you have developed a side effect, such as muscle pain or weakness, you can contact the research team on 0115 8231937.

**What are the possible disadvantages and risks of taking part?**

Occasionally the inhalation of salt solution to induce sputum can make you wheezy. This can be quickly reversed using a salbutamol (Ventolin) inhaler which we will have to hand. The test is done frequently in the department by experienced members of staff.

If you have private medical insurance please check with the company, before agreeing to take part in the trial, whether participation is considered a 'material fact' that should be reported. You will need to do this to ensure that your participation will not affect your medical insurance.

**What are the possible benefits of taking part?**

There are no short term benefits anticipated with this study. By participating, you will be helping us to collect information to see whether statins, particularly simvastatin, could help in the management of patients with COPD, in the future.

**This completes part 1. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.**

## **Part 2 of the information sheet**

### **What if relevant new information becomes available?**

Sometimes we get new information about the treatment being studied during the time you are involved in the study. If this happens, your research doctor will tell you about it and discuss whether you should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you may be asked to sign an agreement outlining the discussion (an updated consent form).

It is also a possibility that on receiving new information your research doctor might consider it to be in your best interests that you should withdraw from the study. He/she will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, we will tell you and explain why, and arrange your continuing care.

### **What will happen if I don't want to carry on with the study?**

You can withdraw from the study at any time if you no longer wish to take part. Information already collected may still be used. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

### **What if there is a problem?**

#### Complaints:

If you have any concerns about the study you should speak with the researchers who will do their best to answer your questions (0115 82 31317). Any concerns or complaints about the way you have been treated during the study or any possible harm you might suffer will be addressed. If you are unhappy and wish to complain formally you can do so through the NHS complaints procedure. Details can be obtained from the hospital.

#### Harm:

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation against the University of Nottingham but you may have to pay your legal costs. The normal National Health Service complaints mechanism will still be available to you.

### **What happens when the research study stops?**

Throughout the study, your care will remain with your GP. If you are also under the hospital, that care will remain in place. At the end of the study, all visits to the research centre will finish and care will revert to your usual doctor. When the research study stops you will continue to take your normal COPD medication and the study medication – simvastatin – will stop.



**Will my taking part in this study be kept confidential?**

Yes. We will follow ethical and legal practice and all information collected about you during the course of the research will be handled in strict confidence.

**Involvement of the General Practitioner/Family doctor (GP)**

With your permission we will write to your GP and notify them that you are going to be taking part in this study.

**What will happen to any samples I give?**

The samples will be collected and analysed and then stored in a secure area, with only direct researchers having access. Samples will be labelled with a code that will allow us to work out that the samples came from you when we get the results, but will ensure that no one outside the research group can identify you. If any of these results could benefit your treatment outside of this study we would be able to share the information with other healthcare professionals involved in your care with your agreement. Samples will be stored until the end of the study and when all analysis is complete, and then they will be destroyed in accordance with the Human Tissue Authority's code of practice.

**Will any genetic tests be done?**

No.

### **What will happen to the results of the research study?**

The results of the paper will be published in a medical journal and may be presented at a meeting or conference. It will not be possible to identify individual people's results in any report or publication. A summary of the results will be available to you should you wish.

### **Who is organising and funding the research?**

The study is being funded by the National Institute of Health Research (NIHR) Respiratory Biomedical Research Unit, which is part of Nottingham University Hospitals NHS Trust and the University of Nottingham.

Please note the research team are not being paid for including you in this study.

### **Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham Research Ethics Committee.

### **Further information and contact details**

Michelle John

Charlotte Bolton

0115 82 31933

0115 82 31317

michelle.john@nottingham.ac.uk

charlotte.bolton@nottingham.ac.uk

## Appendix 2 – Consent Form for the Statin RCT



Nottingham University Hospitals   
NHS Trust

### CONSENT FORM (Version 1.3 02-03-10)

**Title of Study:** The Cardiovascular and Inflammatory Effects of Statin Therapy  
in Patients with COPD

**REC ref:** 10/H0408/10

**Name of Researcher:** Dr C Bolton and Miss M John

**Name of Participant:**

**Please initial box**

1. I confirm that I have read and understand the information sheet version number 1.3 dated 02-03-10 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐
4. I understand and agree that a blood, sputum and urine sample will be taken for analysis of inflammation, cholesterol levels and other tests mentioned in the information sheet. Samples will be stored throughout the study and until analysed and then will be destroyed. ☐
5. I agree to my GP being informed of my participation in this study, and results of any standard blood tests if further action is required. ☐
6. I agree to take part in the above study. ☐

_____ Name of Participant	_____ Date	_____ Signature
_____ Name of Person taking consent (if different from Principal Investigator)	_____ Date	_____ Signature
_____ Name of Principal Investigator	_____ Date	_____ Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Version 1.3 02-03-2010



## ***Do you have COPD?***

### ***Are you interested in taking part in a research study?***

We are seeking volunteer men and women aged 45 – 80 years old to participate in a study based at Nottingham Respiratory Biomedical Research Unit (BRU), City Hospital, Nottingham.

#### **The Cardiovascular and Inflammatory Effects of Statin therapy in patients with COPD**

Chronic obstructive pulmonary disease (COPD) is a common lung condition affecting nearly a million people in the UK. COPD is due to the lungs being damaged and results in breathing difficulties. Drugs currently used for COPD are focused on opening up the narrowed airways. We are researching whether Simvastatin (a drug used to lower cholesterol in the blood) can be used to lower inflammation and therefore lower the risk of heart disease in people with COPD.

The study involves 3 visits over an 8 week period.

**If you would be interested in more information, please contact Dr Bolton or Michelle John at the BRU on:0115 8231933**

**DO YOU SMOKE?**  
**or have you previously?**

**DO YOU HAVE BREATHING PROBLEMS?**

We are seeking volunteer men and women aged 45 – 80 years old to participate in a study based at Nottingham Respiratory Biomedical Research Unit (BRU), City Hospital, Nottingham.

*'The Cardiovascular and Inflammatory Effects of Statin therapy in patients with COPD'*

**If you would be interested in more information, please contact Dr Bolton or Michelle John at the BRU on:**

**0115 82 31933**

**Appendix 4 – Patient Information Sheet for ‘The association of lung function and cardiovascular risk’ Study**

**PATIENT INFORMATION SHEET** version 1.3 (04/01/2011)

**Title: The association of lung function and cardiovascular risk**

**Chief Investigator: Dr Charlotte Bolton**

**Introduction**

You are being invited to take part in a research study. Before you decide, it is important for you to understand why this research study is being conducted and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Please feel free to ask us if there is anything that is not clear or if you require any further information. You may keep this information for future reference.

**Purpose of the Study**

Previous studies have shown that people with poor lung function (who often have chronic lung disease) have an increased risk of cardiovascular disease (problems with the blood vessels) over and above the effects of smoking. However, at the moment we have a limited understanding of the reasons behind this.

Researchers wish to study people with and without lung disease who currently smoke or have smoked in the past, to explore people's risk of developing cardiovascular disease. Researchers will measure the stiffness of people's blood vessels and also look at levels of inflammation in the blood and compare this with people's lung function. Finally, researchers wish to see if people's kidneys are

affected by the stiffening of the hearts main artery; the aorta. It is hoped that by studying people's blood vessels and lung function, it will lead to a greater understanding of why people with poor lung function (who often have chronic lung disease) have an increased risk of cardiovascular disease.

### **Why have I been chosen?**

You have been chosen because you:-

- Have been diagnosed with COPD and are attending one of the researchers Respiratory Out-Patient Clinics.
- Have previously consented to be included on the Nottingham Respiratory Research Database or Professor Ian Halls Volunteer Database and to contacted regarding future research studies.
- Are replying to a poster or advertisement seeking volunteers.

Please note; this study is only available to people of European Ancestry, as the tests used in this study are not valid in any other skin type at present.

### **Do I have to take part?**

It is entirely up to you whether or not you decide to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form (you will be given a copy of this as well). If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw, or a decision not to take part, will not affect the standard of care you already receive.

## **What will happen to me if I take part?**

You will be asked to attend for one study visit at the Respiratory Biomedical Unit at Nottingham City Hospital. During this time (approximately 90 minutes) you will need to have various tests/procedures.

### **Visit 1**

If after reading this information sheet and talking to a member of the research team, you would like to take part in this study, we would ask you to sign a consent form.

In addition we would like to conduct the following tests/procedures:-

- **Blood Sample:** we would like to take a small amount of blood (25mls or the equivalent of 5 teaspoons) from your arm for further analysis.
- **Urine Analysis:** we would like to take a sample of urine (10mls or the equivalent of 2 teaspoons), to analyse certain molecules.
- **Questionnaires:** You will be asked to fill out 3 short questionnaires about how your health affects your daily life.
- **Spirometry Test** (blowing test); which is used to measure your lung function (the size of your lungs and how quickly you can empty them). You will be asked to breathe in and then blow out very fast into a mouth piece.
- **Carbon Monoxide Test:** we would like to measure the level of a gas called carbon monoxide exhaled in your breath. This involves blowing into a machine which is a bit like an alcohol breathalyser to record the concentration of carbon monoxide.
- **Pulse Oximetry;** a simple non-invasive test which measures oxygen saturation in your blood.
- **Blood Pressure**



- **Arterial Stiffness Measurements:** A non-invasive test which involves placing a blood pressure cuff around the participant's upper leg and arm to measure the speed of pulsation down the participant's body, which is related to the stiffness of the arteries. Please be aware that all of the tests listed above will be performed by competent staff following standard operating procedures. Before starting any of the tests we will ensure that there are no reasons for the test not to be performed.
- **Skin Elasticity Measurements:** A non-invasive test used to measure the mechanical properties of people's skin (skin elasticity). Skin elasticity will be measured by applying a small suction force to the skin and measuring how the skin responds to this force.
- **Skin AGE Measurements:** A non-invasive test used to measure people's skin metabolism. This involves participants placing their arm onto the device, which then shines light onto the skin. The reflected light is then measured and analysed.

Please be aware that all of the tests listed above will be performed by competent staff following standard operating procedures. Before starting any of the tests we will ensure that there are no reasons for the tests not to be performed.

Finally, we would like to record your past medical history; including your current medication and smoking history.

### Follow-Up (Optional)

With your consent we would like to contact you in the future to follow you up and perform some of these tests again, in order to create a longitudinal study (a study over a longer period of time) of cardiovascular risk in patients with and without lung disease, dependent on funding. Please be aware that a member of the study team will contact your GP to check your ongoing health status before contacting you.

### **What do I have to do?**

You should continue to carry on with your normal daily activities and take your usual medication. We also ask that you attend the scheduled study visit (although there is some flexibility in terms of the days and times when this can occur) and complete the study paperwork. You may be asked to refrain from eating or drinking for six hours prior to your appointment, although your study doctor will advise you before you attend for your visit.

### **Will any genetic tests be carried out? (Optional)**

Yes; it is hoped that the genetics part of the study (looking for differences in people's genes) will lead to a greater understanding of the relationship between lung function and the blood vessels. Please be aware that the results of any genetic testing will be kept strictly confidential and will NOT be sent out to you as they do not have any clinical relevance and will not affect any private medical or life insurance policies you may have. Please note; this part of the study is **optional** and if you agree to take part in the genetics part of the study you will be asked to sign an optional clause on the consent form.

**What are the possible benefits of taking part?**

You will not benefit directly from taking part in this study, however, it is hoped that the results of this study will lead to a greater understanding of the relationship between lung function and cardiovascular disease.

**What are the possible disadvantages/risks of taking part?**

As with all tests/procedures some people experience side effects, some of which are detailed below:-

**Blood Tests:** Occasionally, some people feel faint during a blood test. If this occurs, please tell the person doing the test, as you should immediately lie down to prevent fainting. Sometimes after donating blood, a bruise develops where the needle was inserted.

**Will travel expenses be reimbursed?**

Participants will not be paid an inconvenience allowance to participate in the study. However, we will cover the cost of travelling to the hospital (maximum £10 allowance per visit).

**What will happen if I don't want to carry on with the study?**

You are free to withdraw from this study at any time and without giving a reason. A decision to withdraw, will not affect the standard of care you already receive. However, please be aware, that should you wish to withdraw, the information collected so far cannot be erased and may still be used in the final project analysis. Any stored tissue samples that can still be identified as yours will be destroyed if you wish.

**Where will my data be stored and will my details be kept confidential?**

All data collected during the period of the study will be stored securely (password protected) on a dedicated trial database. Only the minimum required information for the purposes of the study shall be collected and this information will be subject to the same degree of confidentiality as your NHS notes. Access to the information will be limited to the research team and relevant regulatory authorities.

**Information on the storage and use of tissue samples for research**

Any tissue/blood sample you donate will be stored in a secure research facility at the University of Nottingham (Respiratory Biomedical Research Unit), for as long as is required for the purposes of this study. Your sample will have your code which is unique to yourself, a barcode and date of study. By using these numbers, we can trace which sample belongs to you. The analysis of samples will take place within the Respiratory Biomedical Research Unit at Nottingham City Hospital. Please note; your sample will not be sold for profit or used in any animal research.

Your tissue/blood sample will be retained for 7 years in accordance with the University's Code of Research Conduct and then destroyed should you wish to do so. However, with your permission we would like to retain any remaining tissue/blood in a link-anonymised form for future laboratory research into respiratory disease (as yet unspecified). If you agree, the remaining tissue/blood will be stored on University premises under our Human Tissue Authority License.

**Involvement of the General Practitioner/Family Doctor (GP)**

With your permission we will write to your GP to notify them that you are going to take part in this study, and also inform them of any abnormal test results (if any) for appropriate action.

**Who is organising and funding this study?**

The research has been organised and funded by the University of Nottingham. Please be aware that the research team involved in this study are not being paid for including you in this study.

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable ethical opinion for conduct in the NHS by the Leicestershire, Northamptonshire & Rutland Research Ethics Committee 1 and will be subject to the Data Protection Act.

**What will happen to the results of this study?**

We intend to publish the results of this study in a medical respiratory journal. A summary of these results will also be made available on the Nottingham Respiratory Biomedical Research Unit's website ([www.nrbru.org.uk](http://www.nrbru.org.uk)). If you wish, you will be informed of the study results in clinic by the research nurse or doctor. Furthermore, a copy of any published material regarding the study will be made freely available to you.

**What if there is a problem?**

If you wish to complain or have any concerns about the way in which you have been treated, please get in touch with the research team (see below), who will do their best to answer any problems you might have. In addition, the normal NHS complaints procedures are also available to you (e.g. Patient Advice and Liaison Service – PALS). Details can be obtained from the hospital.

### **Contact for Further Information**

Dr Charlotte Bolton

Tel: 0115 82 31317

E-Mail: [charlotte.bolton@nottingham.ac.uk](mailto:charlotte.bolton@nottingham.ac.uk)

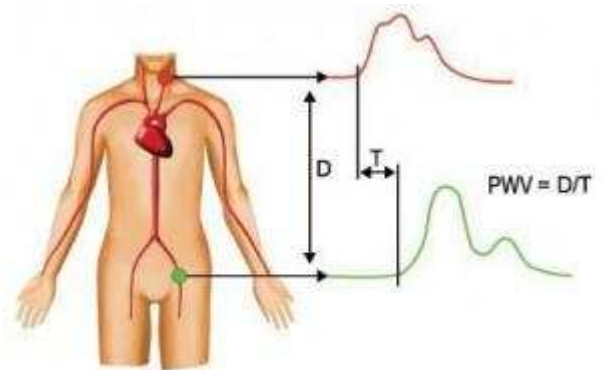
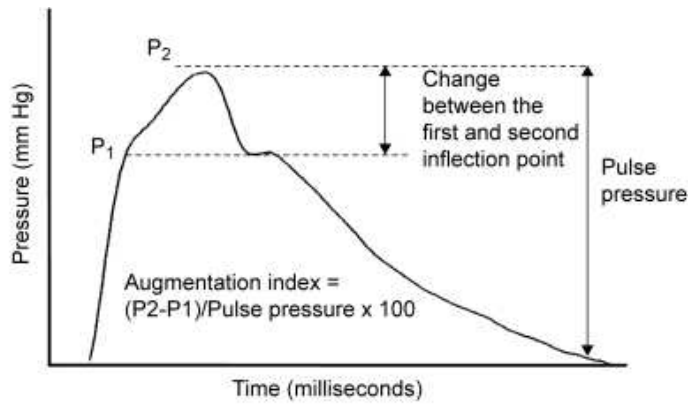
Or

Michelle John

Tel: 0115 82 31933

E-Mail: [michelle.john@nottingham.ac.uk](mailto:michelle.john@nottingham.ac.uk)

## Appendix 5 – Waveforms for Augmentation Pressure (AP) and Alx



$P_1$  = first point of inflection

$P_2$  = second point of inflection

$D$  = distance covered by the waves – measured as the surface distance between the two recording sites

$T$  = time measured between the feet of the two waveforms

## Appendix 6 - Diagram for Path Length Measurements for Arterial Stiffness Tests

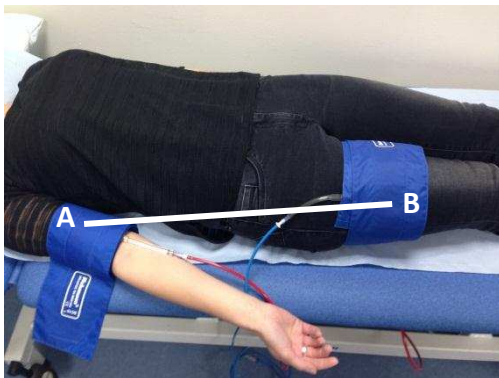
i



**Key -** ● carotid ● supra sternal notch ● femoral ● radial

Pulse and measurement locations used for sphygmocor arterial stiffness measurements

ii



iii



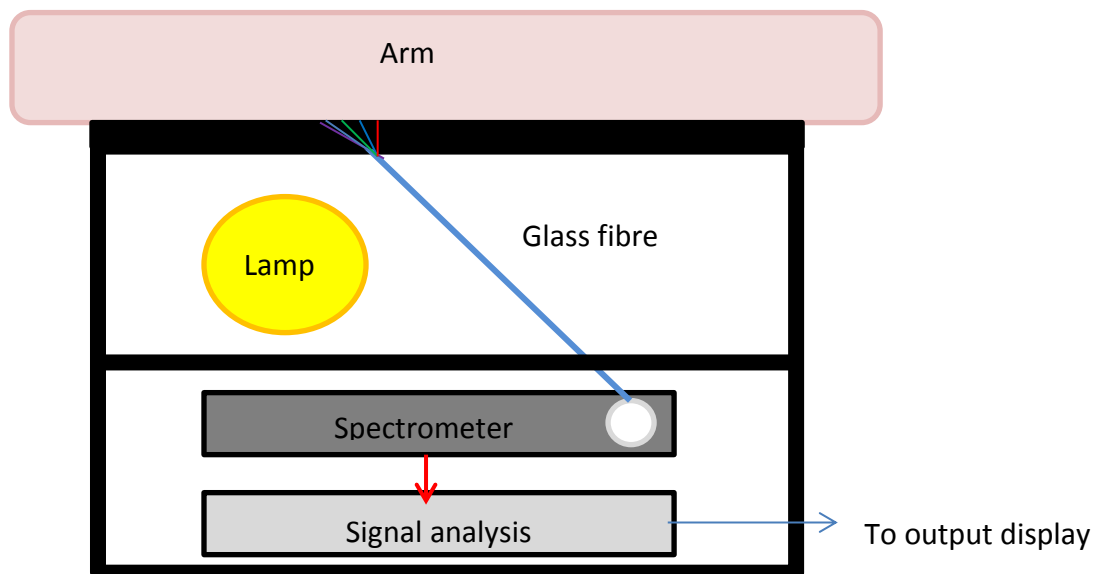
In picture ii and iii measurements for path length are taken from points A to B – brachial to femoral and carotid to femoral respectively for arterial stiffness measurements using the vicorder.



## Appendix 7 – Skin AGE Reader Diagrams



### AGE Reader Schematic Diagram



## **Appendix 8 – MRC Dyspnoea Scale**

### **Medical Research Council Dyspnoea Scale**

1. Not troubled by breathlessness except on strenuous exercise
2. Short of breath when hurrying or walking up a slight hill
3. Walks slower than contemporaries on the level because of breathlessness, or has to stop for breath when walking at own pace
4. Stops for breath after about 100 m or after a few minutes on the level
5. Too breathless to leave the house, or breathless when dressing or undressing

## Appendix 9 – COPD Assessment Test

Your name:

Today's date:



### How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional measure the impact COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers, and test score, can be used by you and your healthcare professional to help improve the management of your COPD and get the greatest benefit from treatment.

For each item below, place a mark (X) in the box that best describes you currently. Be sure to only select one response for each question.

**Example:** I am very happy ☐ 0 ☒ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 I am very sad

		SCORE
I never cough	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 I cough all the time	<input type="text"/>
I have no phlegm (mucus) in my chest at all	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 My chest is completely full of phlegm (mucus)	<input type="text"/>
My chest does not feel tight at all	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 My chest feels very tight	<input type="text"/>
When I walk up a hill or one flight of stairs I am not breathless	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 When I walk up a hill or one flight of stairs I am very breathless	<input type="text"/>
I am not limited doing any activities at home	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 I am very limited doing activities at home	<input type="text"/>
I am confident leaving my home despite my lung condition	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 I am not at all confident leaving my home because of my lung condition	<input type="text"/>
I sleep soundly	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 I don't sleep soundly because of my lung condition	<input type="text"/>
I have lots of energy	<input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 I have no energy at all	<input type="text"/>
		<b>TOTAL SCORE</b> <input type="text"/>

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## Appendix 10 – St George's Respiratory Questionnaire

### **ST. GEORGE'S RESPIRATORY QUESTIONNAIRE ORIGINAL ENGLISH VERSION**

#### **ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)**

*This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.*

*Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.*

*Before completing the rest of the questionnaire:*

*Please tick in one box to show how you describe your current health:*

Very good	Good	Fair	Poor	Very poor
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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UK/ English (original) version

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*continued...*

## St. George's Respiratory Questionnaire PART 1

**Questions about how much chest trouble you have had over the past 4 weeks.**

Please tick (✓) one box for each question:

- |   | most<br>days<br>a week   | several<br>days<br>a week | a few<br>days<br>a month | only with<br>chest<br>infections | not<br>at<br>all         |
|---|--------------------------|---------------------------|--------------------------|----------------------------------|--------------------------|
| 1. Over the past 4 weeks, I have coughed:   | <input type="checkbox"/> | <input type="checkbox"/>  | <input type="checkbox"/> | <input type="checkbox"/>         | <input type="checkbox"/> |
| 2. Over the past 4 weeks, I have brought up phlegm (sputum):  | <input type="checkbox"/> | <input type="checkbox"/>  | <input type="checkbox"/> | <input type="checkbox"/>         | <input type="checkbox"/> |
| 3. Over the past 4 weeks, I have had shortness of breath:   | <input type="checkbox"/> | <input type="checkbox"/>  | <input type="checkbox"/> | <input type="checkbox"/>         | <input type="checkbox"/> |
| 4. Over the past 4 weeks, I have had attacks of wheezing:   | <input type="checkbox"/> | <input type="checkbox"/>  | <input type="checkbox"/> | <input type="checkbox"/>         | <input type="checkbox"/> |
| 5. During the past 4 weeks, how many severe or very unpleasant attacks of chest trouble have you had? |                          |                           |                          |                                  |                          |

Please tick (✓) one:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?  
(Go to question 7 if you had no severe attacks)

Please tick (✓) one:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 4 weeks, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) one:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) one:

- No ☐
- Yes ☐

## St. George's Respiratory Questionnaire PART 2

### Section 1

How would you describe your chest condition?

Please tick (✓) one:

- The most important problem I have ☐  
 Causes me quite a lot of problems ☐  
 Causes me a few problems ☐  
 Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) one:

- My chest trouble made me stop work altogether ☐  
 My chest trouble interferes with my work or made me change my work ☐  
 My chest trouble does not affect my work ☐

### Section 2

**Questions about what activities usually make you feel breathless these days.**

Please tick (✓) in **each box** that applies to you **these days**:

	True	False
Sitting or lying still	<input type="checkbox"/>	<input type="checkbox"/>
Getting washed or dressed	<input type="checkbox"/>	<input type="checkbox"/>
Walking around the home	<input type="checkbox"/>	<input type="checkbox"/>
Walking outside on the level	<input type="checkbox"/>	<input type="checkbox"/>
Walking up a flight of stairs	<input type="checkbox"/>	<input type="checkbox"/>
Walking up hills	<input type="checkbox"/>	<input type="checkbox"/>
Playing sports or games	<input type="checkbox"/>	<input type="checkbox"/>

## St. George's Respiratory Questionnaire PART 2

### Section 3

**Some more questions about your cough and breathlessness these days.**

Please tick (✓) in **each box** that applies to you **these days**:

	True	False
My cough hurts	<input type="checkbox"/>	<input type="checkbox"/>
My cough makes me tired	<input type="checkbox"/>	<input type="checkbox"/>
I am breathless when I talk	<input type="checkbox"/>	<input type="checkbox"/>
I am breathless when I bend over	<input type="checkbox"/>	<input type="checkbox"/>
My cough or breathing disturbs my sleep	<input type="checkbox"/>	<input type="checkbox"/>
I get exhausted easily	<input type="checkbox"/>	<input type="checkbox"/>

### Section 4

**Questions about other effects that your chest trouble may have on you these days.**

Please tick (✓) in **each box** that applies to you **these days**:

	True	False
My cough or breathing is embarrassing in public	<input type="checkbox"/>	<input type="checkbox"/>
My chest trouble is a nuisance to my family, friends or neighbours	<input type="checkbox"/>	<input type="checkbox"/>
I get afraid or panic when I cannot get my breath	<input type="checkbox"/>	<input type="checkbox"/>
I feel that I am not in control of my chest problem	<input type="checkbox"/>	<input type="checkbox"/>
I do not expect my chest to get any better	<input type="checkbox"/>	<input type="checkbox"/>
I have become frail or an invalid because of my chest	<input type="checkbox"/>	<input type="checkbox"/>
Exercise is not safe for me	<input type="checkbox"/>	<input type="checkbox"/>
Everything seems too much of an effort	<input type="checkbox"/>	<input type="checkbox"/>

### Section 5

**Questions about your medication, if you are receiving no medication go straight to section 6.**

Please tick (✓) in **each box** that applies to you **these days**:

	True	False
My medication does not help me very much	<input type="checkbox"/>	<input type="checkbox"/>
I get embarrassed using my medication in public	<input type="checkbox"/>	<input type="checkbox"/>
I have unpleasant side effects from my medication	<input type="checkbox"/>	<input type="checkbox"/>
My medication interferes with my life a lot	<input type="checkbox"/>	<input type="checkbox"/>

## St. George's Respiratory Questionnaire PART 2

### Section 6

*These are questions about how your activities might be affected by your breathing.*

Please tick (✓) in **each box** that applies to you **because of your breathing**:

	True	False
I take a long time to get washed or dressed	<input type="checkbox"/>	<input type="checkbox"/>
I cannot take a bath or shower, or I take a long time	<input type="checkbox"/>	<input type="checkbox"/>
I walk slower than other people, or I stop for rests	<input type="checkbox"/>	<input type="checkbox"/>
Jobs such as housework take a long time, or I have to stop for rests	<input type="checkbox"/>	<input type="checkbox"/>
If I walk up one flight of stairs, I have to go slowly or stop	<input type="checkbox"/>	<input type="checkbox"/>
If I hurry or walk fast, I have to stop or slow down	<input type="checkbox"/>	<input type="checkbox"/>
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	<input type="checkbox"/>	<input type="checkbox"/>
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	<input type="checkbox"/>	<input type="checkbox"/>
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	<input type="checkbox"/>	<input type="checkbox"/>

### Section 7

*We would like to know how your chest usually affects your daily life.*

Please tick (✓) in **each box** that applies to you **because of your chest trouble**:

	True	False
I cannot play sports or games	<input type="checkbox"/>	<input type="checkbox"/>
I cannot go out for entertainment or recreation	<input type="checkbox"/>	<input type="checkbox"/>
I cannot go out of the house to do the shopping	<input type="checkbox"/>	<input type="checkbox"/>
I cannot do housework	<input type="checkbox"/>	<input type="checkbox"/>
I cannot move far from my bed or chair	<input type="checkbox"/>	<input type="checkbox"/>



## St. George's Respiratory Questionnaire

*Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):*

- Going for walks or walking the dog
- Doing things at home or in the garden
- Sexual intercourse
- Going out to church, pub, club or place of entertainment
- Going out in bad weather or into smoky rooms
- Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

.....

.....

.....

.....

Now would you tick in the box (one only) which you think best describes how your chest affects you:

- It does not stop me doing anything I would like to do ☐
- It stops me doing one or two things I would like to do ☐
- It stops me doing most of the things I would like to do ☐
- It stops me doing everything I would like to do ☐

*Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.*

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